Executive Summary

On August 30, 1999, the South Florida Water Management District (District) contracted with DB Environmental Laboratories, Inc. (DBEL) to perform a 100-week evaluation of Submerged Aquatic Vegetation/Limerock (SAV/LR) Treatment System technology for reducing phosphorus (P) discharge from Everglades Agricultural Area (EAA) waters. The objectives of this project are to assess the long-term, sustainable performance of this technology, and to develop design and operational criteria for a full-scale SAV/LR system. For this effort, we are performing scientific and engineering work at Stormwater Treatment Area (STA)-1W at several spatial scales: outdoor microcosms and mesocosms, test cells (0.2 ha), Cell 4 (146 ha) and Cell 5 (1,100 ha). This document is a quarterly progress report describing work efforts of DBEL's project team from February – April 2000. Key accomplishments and findings are as follows.

During this quarter, we continued using existing mesocosms at both the North and South Advanced Treatment Technology (NATT and SATT) sites of STA-1W to assess effects of hydraulic loading rates, water type (Post-BMP vs. Post-STA), and system configuration on SAV/LR P removal performance.

We developed protocols and performed preliminary experimentation in preparation for a microcosm study to assess the effects of calcium, alkalinity and soluble reactive P levels on SAV P removal performance. This experiment will facilitate our understanding of P removal processes in SAV systems, and will improve our ability to predict SAV wetland performance as a function of the water chemistry fluctuations that commonly occur in the Everglades Agricultural Area runoff.

During April, we established protocols for a fluctuating water depth study. In this experiment, water column depths for SAV mesocosms that had been operated at steady state depths ranging from 0.4 to 1.2 m will be varied in a cyclical fashion. In the first experiment, the depths of the 0.4 m deep mesocosms will be raised to 0.8 m, and the depths of the 1.2 m deep mesocosms will be reduced to 0.8 m. Phosphorus removal performance of these systems will be measured under this modified depth regime for 4 to 6 weeks. Depth fluctuation experiments will be performed through October 2000.

We performed spatial and temporal water quality sampling in "non-harvested" and previously harvested mesocosms prior to termination of the SAV "harvest" experiment. Biomass density of SAV species also was measured upon completion of the study. Relative to non-harvested systems, previously harvested mesocosms exhibited slightly lower SRP concentrations in portions of the water column, and slightly higher pH and D.O. concentrations. Harvesting also affected SAV speciation, favoring increased biomass of the macrophyte *Potamogeton*.

A pulse loading study was initiated using SAV mesocosms that previously had received hydraulic loading rates (HLRs) of 11, 22 and 55 cm/day. We established a loading schedule for the mesocosms that is similar to the "STA-2" hydraulic loading data set in the following respects: our protocol has the same average seasonal flow patterns; the same percentage of "no-flow" weeks; and, the same standard deviation on a seasonal basis. We also are scaling (increasing) the STA-2 HLRs by factors of 5X, 10X and 25X, with flow provided to the mesocosms in two-week long pulses. These increased flows were selected to match the prior, high HLRs (11 – 55 cm/day) to the SAV mesocosms, which should provide continuity with previous HLR studies.

We continued long-term monitoring of shallow (0.09 m deep) SAV/periphyton/LR raceways that receive Post-STA waters, as well as the deeper (0.4 m) tanks containing SAV cultured on muck, sand and limerock substrates. Phosphorus removal performance by SAV was similar among all substrates during this quarter.

In the 0.2 ha test cells, we interrupted operations to install limerock berms in south test cell (STC)-9 and north test cell (NTC)-15. We also initiated efforts to remove the aquatic macrophyte *Hydrilla verticillata* from STC-4 and STC-9.

In STA-1W Cell 5, we performed two experiments on inoculation techniques for SAV (*Najas*, *Ceratophyllum*, *Chara*) and we established 120 monitoring stations to evaluate how quickly SAV spreads internal to the 930 ha western portion of the wetland. As of February 2000, *Ceratophyllum* was the most prominent SAV species, occurring at 64 of 120 sampling locations

within the wetland. *Najas* was found at 17 stations, but *Chara* at none. Based on our field observations at the 120 station monitoring network, the standing crop biomass of SAV at most sites within Cell 5 remains extremely low.

During this quarter, we continued reviewing historic STA-1W Cell 4 performance data. Cell 4, which is dominated by *Najas* and *Ceratophyllum*, continued to provide exemplary P removal performance through the end of 1999. Cell 4 outflow TP concentrations in both 1998 and 1999 averaged 14 µg/L. The P mass removal rate for Cell 4 during this 24 month period averaged 1.5 g P/m^2-yr.

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Introduction

On August 30, 1999, the District contracted with DB Environmental Laboratories, Inc. (DBEL) to design, construct, operate, and evaluate a 100-week, multi-scale demonstration of SAV/Lime-rock Treatment System technology for reducing phosphorus (P) discharge from Everglades Agricultural Area (EAA) waters. The objectives of this project are to:

- Design and execute a scientific and engineering research plan for further evaluation of the technical, economic and environmental feasibility of using SAV/LR system for P removal at both the basin and sub-basin scale.

- Obtain samples adequate to conduct a Supplemental Technology Standard of Comparison (STSOC) analysis.

- Provide information and experience needed to design a full-scale SAV/LR system.

This document is the second quarterly progress report describing work efforts during February – April 2000. This report focuses on methodology and findings from the mesocosm experiments (Task 5), Test Cell studies (Task 6), and STA-1W Cell 5 inoculation studies (Task 10). We also have included selected analyses of the historical performance of STA-1W Cell 4 (Task 9).

Task 5. Mesocosm Investigations

During February-April 2000, we continued using existing mesocosms at both the North and South Advanced Treatment Technology (NATT and SATT) sites of STA-1W to assess effects of hydraulic loading rates, water type (Post-BMP vs. Post-STA), and system configuration on P removal performance. Activities are listed below by experimental subtask.

Effects of Calcium/Alkalinity and Soluble Reactive Phosphorus Concentrations on Phosphorus Coprecipitation (Subtask 5i)

Findings from our Phase I research using Post-BMP waters suggest that P removal in an SAV system is controlled in part by water column hardness and alkalinity. In April, we began establishing and refining the experimental approach to the first two experiments embodied in Subtask 5i.

This experiment was performed by first pre-conditioning the submerged macrophyte *Najas* to both "P-enriched" and "P-starved" conditions. We conditioned *Najas* to "P-enriched" status by repeatedly (every three days) adding SRP to a growth medium to yield a final SRP concentration of ~200 µg/L. Amendments of SRP were withheld from the growth medium to create "P-starved" *Najas*. The conditioning period lasted 35 days. The first experiment will entail subjecting "P-enriched" and "P-starved" *Najas* to a constant SRP concentration at high and low levels of calcium and alkalinity over a two-day measurement period to discern the relative importance of P uptake by SAV vs. coprecipitation of P in the water column.

Various screening runs were completed in March and April, which identified the optimum plant stocking densities (ratio of water volume to wet weight of SAV), source waters, nutrient amendments, temperature ranges, light regimes and attainable pH values. The trials also explored the need for, and methods of, dechlorinating and stripping phosphorus from the tap water to be used as the initial growth medium. Tap water will be used as the water source because of its low Ca (30 mg/L) and alkalinity (56 mg $CaCO_3$/L), relative to agricultural drainage waters ("typical" Post-BMP concentrations of 71 mg Ca/L and 227 mg $CaCO_3$/L). We also collected preliminary data on SRP uptake and release by *Najas*, precision within replicates,

and source water characterization. Based on these preliminary trials, we anticipate proceeding with the definitive experiment in May.

Fluctuating Water Depth Mesocosms (Subtask 5ii)

The fluctuating water depth experiment is scheduled to start mid-May, with a reduction of the water depth to 0.8 m deep in duplicate deep (1.2 m) mesocosms at the NATTS. Additionally, the water depth in two shallow (0.4 m) mesocosms will be increased to 0.8 m. These depths will be maintained for 5 weeks, after which time the mesocosms will be returned to the original depths of 0.4 m and 1.2 m. This 5-week cycle of fluctuating depths will be repeated four times during the experiment. For the fifth and final 5-week period, we will reduce the depth of the two shallow mesocosms to 0.15 m while the water depth of the deep mesocosms will be at 0.8 m. One of each the shallow and deep mesocosms and each of the three moderate depth mesocosms will remain at a constant depth for "control" comparisons.

Currently, the nine mesocosms at the NATTS are sampled for total P and SRP weekly to provide a background performance level prior to water depth fluctuation. Throughout April, the influent TP concentration ranged from 49 – 147 µg/L. Effluent concentrations were similar among the three depths (27 – 36 µg TP/L). The 21 – 177 µg/L SRP range ($\bar{x}$ = 54 µg/L) for influent concentrations decreased to an average effluent value of 3 µg/L with no discernible differences among depth treatments.

Harvest Study (Subtask 5iii)

Two major investigations were completed during the February-April quarter using the "Harvest" mesocosms. A Spatial-Temporal profile was performed to observe changes over the diurnal cycle in the water column chemistry with depth, and a final biomass harvest was performed just prior to termination of the experiment, which documented changes in the SAV community composition. The results of these efforts are discussed below.

Spatial-Temporal Profile

We had previously removed SAV biomass from the three replicate "harvested" tanks on August 19, 1999, by clipping and discarding the SAV occupying the top half of the water

column. Phosphorus data collected subsequent to the harvest indicated that the P removal efficiency in the mesocosms was adversely affected, but then returned to near pre-harvest levels after a 7-week "recovery" period.

We performed a Spatial-Temporal study on February 7 and 8, 2000, with the intent of examining at a finer spatial and temporal resolution the effects that harvesting may have had on the chemical processes in a post-harvest, but "fully recovered" SAV system. For an unharvested "control", we sampled duplicate, long hydraulic retention time (HRT) mesocosms scheduled to be used later in the Pulse Loading and Long-Term Monitoring tasks. These tanks shared similar depths (80 cm), hydraulic loading rates (HLR: 10-11 cm/day) and hydraulic retention times (HRT: 7-8 days) with the harvested tanks.

Water samples were collected and field measurements (pH, D.O., redox potential, and temperature) recorded from three depths (3, 30, and 60 cm) at both influent and effluent ends of duplicate mesocosms from the harvested and non-harvested treatments. These measurements were performed during the afternoon (1435-1700) and early morning (0500-0700) on February 7-8, 2000. Water samples were returned to the laboratory and analyzed for soluble reactive P (SRP).

Mean SRP concentrations of the afternoon and early morning (pre-dawn) inflow Post-BMP water during this sampling event were 63 and 79 µg/L, respectively. The labile nature of SRP in SAV systems is readily seen by the large decreases from the inflow SRP concentrations within the mesocosms (Figure 1). Effluent region SRP concentrations in the unharvested mesocosms were less than the method detection limit (2 µg/L) at all depths, regardless of sampling time. SRP concentrations in the effluent regions of the harvested mesocosms also were low, but above the detection limit.

Within-treatment differences in the SRP concentrations between afternoon and pre-dawn were minimal at all stations, with the only exception being the 60 cm depth at the influent end of the unharvested mesocosms (Figure 1). There were, however, noticeable differences in SRP concentrations as a function of previous harvest history. Compared to the harvested

mesocosms, the unharvested mesocosms had higher SRP concentrations at the influent region, but lower concentrations at the effluent region. The reason for these between-treatment differences in SRP levels is unknown.

Harvesting also appeared to have promoted higher pH values in the water column. Afternoon surface water (3 cm) pH values in the harvested mesocosms were 0.25 to 0.35 units higher than the unharvested controls; there were no pre-dawn differences between the two treatments (Figure 1).

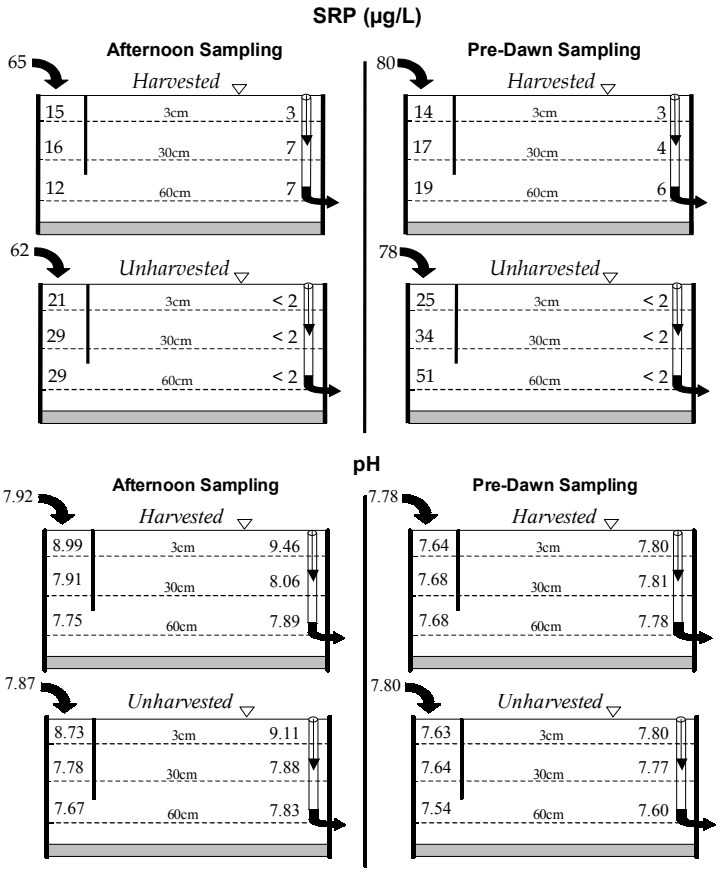

Figure 1. Soluble reactive phosphorus concentrations and pH values during the Spatial-Temporal Study, February 7-8, 2000, in harvested and unharvested mesocosms at the NATTS. Each value within a treatment is a mean of duplicate mesocosms, except for the inflow stations.

As expected, water column dissolved oxygen concentrations varied widely over the diel cycle. Supersaturated conditions, where concentrations exceeded 9.0 mg/L at 21 °C, were detected during the afternoon at the surface of both harvested and unharvested treatments (Figure 2). These high daytime concentrations diminished with depth and during the pre-dawn. At no time or location were the mesocosms anoxic, although the lowest concentration recorded (1.0 mg/L)

indicates that hypoxic conditions did prevail at the 60 cm depth of the unharvested mesocosms in the early morning. The dissolved oxygen concentrations in the unharvested mesocosms were always slightly lower than in the harvested mesocosms at all times and depths (Figure 2), probably due to the higher biomass density (and increased respiration) in the former systems.

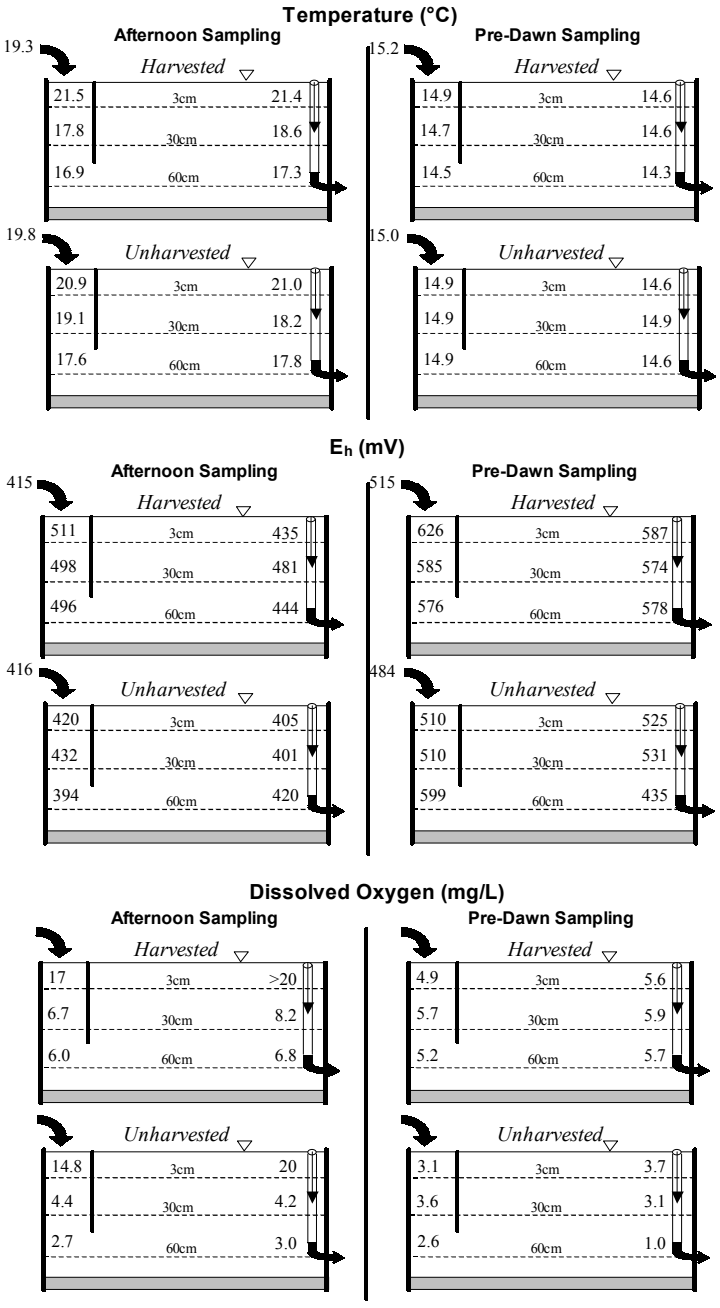

Figure 2. Dissolved oxygen concentrations and redox and temperature values during the Spatial-Temporal Study, February 7-8, 2000, in harvested and unharvested mesocosms at the NATTS. Each value within a treatment is a mean of duplicate mesocosms, except for the inflow stations.

* denotes values from only one of the duplicate mesocosms because of probe malfunction.

Reduction-oxidation potentials (redox) corroborated the dissolved oxygen concentrations in that the oxidation potential in the water column (+394 to +626 mV, Figure 2) was always above the range associated with reducing conditions (+300 to +350 mV). Although still within the oxidized range, the redox potentials from unharvested mesocosms were slightly depressed compared to those from the harvested mesocosms.

Temperature in harvested and unharvested mesocosms for a given depth and sampling time usually differed by less than 1°C (Figure 2). There were, however, vertical temperature gradients during the day in both sets of treatment mesocosms, which then dissipated during the night.

Biomass Harvest

On March 15, 2000 we conducted a final biomass harvest in "closing out" the three triplicate Harvest Study mesocosms. We measured wet weight biomass for each SAV species during this final harvest and dried subsamples at 70–80°C for dry: wet weight ratios. In contrast to the previous harvest (August 19, 1999) where SAV was collected by clipping the SAV occupying the top half of the water column, the harvest methodology for this final harvest was similar to the original harvest performed on September 14, 1998, whereby the entire plant (i.e., including roots) was removed and weighed.

Noticeable species shifts within the SAV community occurred during the 18-month investigation (Figure 3). *Najas* dominated the SAV community in September 1998, comprising 77 ± 6% of the entire dry weight biomass. However, by August of 1999, *Najas* biomass in the three mesocosms decreased to between 23 and 32% ($\bar{x}$ = 28 ± 5%) of the total plant community. The relative distribution of *Najas* among the replicates appeared to stabilize by the March 2000 harvest at 24 ± 1%. *Potamogeton* became more dominant as it increased from only 3% of the total SAV biomass in September 1998 to 20% and 28% in August 1999 and March 2000, respectively. *Chara* biomass also increased from the initial harvest in September 1998 (Figure 3). The distribution of *Ceratophyllum* remained between 12 and 19% during the 18 months following the first harvest. A filamentous alga (periphyton) occurred sporadically in the first two harvests, but increased to nearly 30% of the overall biomass by March 2000.

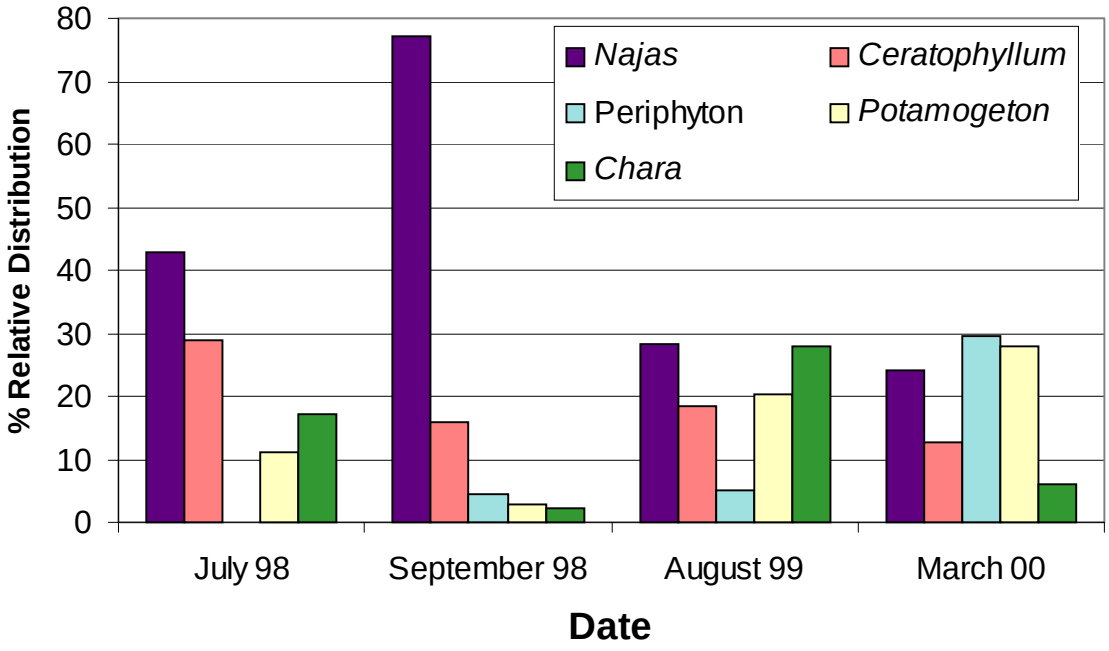

Figure 3. Mean relative distribution (% of total dry weight biomass) of SAV genera for three replicate mesocosms on three harvest dates compared to the initial stocking density. The first harvest, September 14, 1998, was 59 days after initial stocking (July 17, 1998). Whole plants were harvested, weighed and returned to respective mesocosms. During the second harvest (August 19, 1999), 339 days after the first harvest, all plants occupying the top half of the water column were clipped, weighed and discarded. The final harvest occurred March 15, 2000, when whole plants were harvested, weighed and returned to respective mesocosms. Following the third harvest, the experiment was terminated.

Besides harvesting, other factors such as nutritional requirements, seasonal effects and grazing can affect species dominance within the SAV community. We cannot, therefore, state with certainty the exact effects of harvesting since control (unharvested) mesocosms were not sampled at the same times as the harvested mesocosms. Instead, these tanks are serving as the 0.8 m depth "control" treatments of the "Fluctuating Depth Study".

It appears that harvesting may favor growth of *Potamogeton* and *Chara* (for the September 1998 harvest only) to the detriment of *Najas.* Initial harvests undertaken in other mesocosms at the NATTS during the past two years have yielded only minor amounts (< 5%) of *Potamogeton. Chara,* on the other hand, has tended to become a dominant member in those same mesocosms,

suggesting that other factors may be more important than harvest history for this genus. *Ceratophyllum* appears to be unaffected by harvesting. Periphyton biomass increased during the period after the second harvest (August 1999 to March 2000), although this may have been unrelated to harvesting since periphyton abundance fluctuates considerably in the unharvested mesocosms.

Even though harvesting may not be as dominant a factor as nutritional or seasonal effects in determining the composition of the SAV community, the method of harvesting may have played an influential role. For example, we uprooted entire plants and weighed them (wet weight) before returning them to the mesocosms in the first harvest (September 14, 1998), which may have been detrimental to *Najas* with its deeply rooted habit. The clipping performed in the second harvest on August 19, 1999, which left the roots and lower stems intact, may have been more advantageous to *Potamogeton* than *Najas*, perhaps by stimulating *Potamogeton* growth via hormone (i.e. auxin) production such as commonly reported for terrestrial plants (Salisbury and Ross 1978).

Besides the possibility of differential production of growth hormones, enhanced light penetration following harvests may also have played an important role in the dominance of *Chara* (after the September 14, 1998 harvest only) and *Potamogeton* (after both the September 14, 1998 and August 19, 1999 harvests). Steinman et al. (1997) reported that light appeared to be a strong regulator in influencing *Chara* phenology and abundance in Lake Okeechobee. Their data showed that charophyte biomass was inversely related to water depth and positively related to Secchi disk transparency, suggesting that irradiance strongly influences charophyte distribution in the lake. The hypothesis was further supported by laboratory experiments of photosynthetic measurements and photosynthesis irradiance curves: the irradiance at which photosynthesis is initially saturated was an order of magnitude greater than the ambient light reaching the charophyte populations.

Potamogeton also responds to increased light availability by increasing shoot density, and shoot and root biomass (Barko et al. 1982). *Potamogeton* may out-compete *Najas* subsequent to harvesting by quickly forming a canopy with broad leaves, thereby limiting light penetration to

the submersed *Najas* plant. Additionally, *Potamogeton* may have an advantage over *Chara* after harvest because of its storage capacity in root structures, which were disturbed during the first harvest (September 14, 1998), but remained intact after the second harvest (August 19, 1999).

Long-Term Phosphorus Removal Mesocosms (Subtask 5iv)

We have continued to operate SAV/LR mesocosms at 11, 22 and 55 cm/day HLRs since summer of 1998. The lower two HLRs are within the range of HLRs received by Cell 4: between 7 and 28 cm/day based on 3-month rolling averages between May 1, 1995 and April 30, 1999 (Chimney et al. 2000).

The high (~250 µg/L) total P influent levels (post-BMP waters) in January 2000 returned to levels below 100 µg/L by the end of February 2000 (Figure 4). Because of its high HLR (55 cm/day), the outflow from the short retention time mesocosms (1.5 days) closely mirrored the

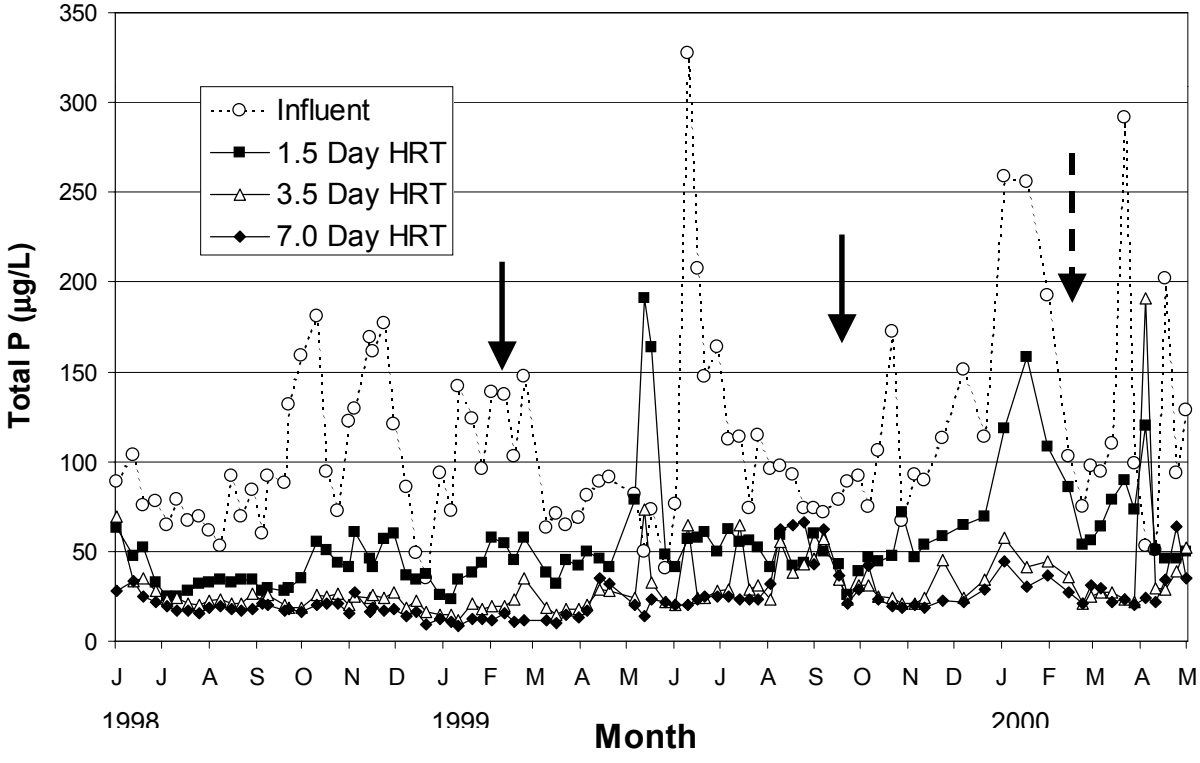

Figure 4. Total phosphorus concentrations in the influent and effluents of 1.5, 3.5 and 7.0-day HRT mesocosms from June 1, 1998 to May 1, 2000. Data from only one mesocosm of each HRT is presented for the period between February 10 and September 29, 1999 (arrows), and after February 23, 2000 (dashed arrow).

fluctuating inflow concentrations. Effluents from the 3.5- and 7.0- day HRT mesocosms exhibited comparable total P concentrations (27 and 26 µg/L), substantially lower than that of the 1.5-day HRT treatment. From the total P concentration data for the quarter (February – April 2000), it appears that a 1.5-day HRT is too short to successfully dampen the occasional spikes in total P concentrations of the post-BMP drainage waters.

Pulse-Loading and Drydown-Reflooding Mesocosms (Subtask 5v)

On February 23, 2000, we initiated the 39-week pulse-loading experiment in six mesocosms at the NATTS. These mesocosms were originally part of Phase I hydraulic loading rate experiments that consisted of triplicate mesocosms receiving treatments of 11, 22, and 55 cm/day HLR's. Six of the nine mesocosms from that experiment were harvested and re-stocked in February 1999 (as part of Phase I close-out efforts); these are the six mesocosms that will receive the pulsed loading. The remaining three mesocosms from Phase I are maintained at constant loadings of 11, 22, and 55 cm/day as controls (in conjunction with Long-Term Monitoring, Subtask 5iv).

We based our pulse-loading schedule for mesocosm experiments on an analysis of Dr. William Walker's STA-2 data set, which is a 9.75-year projected loading scenario that has been adapted by the District for STA design purposes. We analyzed the STA-2 data set on a seasonal basis by dividing each year's data into four 13-week 'seasons'. Each season of each year was analyzed for three parameters: mean seasonal flow, standard deviation of seasonal flow, and percentage of zero-flow weeks. Table 1 shows a comparison of key parameters for our seasonal analysis (on a weekly basis) of the STA-2 data set and our biweekly loading schedule (shown for a 2.4 cm/day HLR).

Table 1. Comparison of STA-2 and DBEL loading schedules.

	Season	STA-2 (analyzed on a weekly basis)	DBEL's Bi-weekly Schedule
% of No-Flow Days	Winter	31%	15%
	Spring	52%	62%
	Summer	13%	15%
	Fall	44%	31%
Average Flow (cm/day)	Winter	1.7	1.8
	Spring	1.7	1.4
	Summer	4.2	4.3
	Fall	1.8	2.1
Standard Deviation (cm/day)	Winter	2.9	1.7
	Spring	3.9	2.2
	Summer	4.7	3.2
	Fall	3.2	2.4

Our pulse-loading schedule for mesocosm experiments is provided in Table 2 and is based on the following premises:

- Each pulse period will last two weeks. This was chosen as a compromise between data collection needs (the longer the time between pulses, the more measurable a flow effect would be) and staying close to the short pulse durations that typify the STA-2 data set.
- DBEL's loading schedule has approximately the same average seasonal flow patterns represented in the STA-2 data set (Table 1).
- DBEL's loading schedule has approximately the same percentage of "no flow" weeks as the STA-2 data set on a seasonal basis (Table 1).
- The standard deviation of flows in DBEL's loading schedule is as close as possible to those in the STA-2 data set on a seasonal basis (Table 1).

We intend to operate the six mesocosms with paired treatments at mean loadings of 11, 22, and 55 cm/day. These HLR's are scaled by factors of approximately 5, 10, and 25, respectively, over the mean 2.4 cm/day HLR inherent in Dr. Walker's STA-2 data set (Table 2). The pulse-loading schedule for the three treatments is identical, except that each treatment applies a different flow scale factor.

During this reporting period (February 20-May 1), there were five two-week periods when the HLRs were changed. During the first two weeks (February 20-March 4), inflows to all six of the mesocosms ceased. Hydraulic loading was resumed for the next two weeks (March 5 -18) at rates of 22, 44, and 110 cm/day to the low, medium, and high loading rate treatment mesocosms, respectively. For the third two-week period, each HLR was reduced to 4.4, 8.8, and 22 cm/day, respectively. During weeks seven and eight, flow was discontinued, and in weeks nine and ten, loadings resumed at 13.2, 26.4, and 66 cm/day for the low, medium and high loading rate treatments, respectively.

Table 2. Forty-week (Feb. 20 - Nov. 27, 2000) pulse loading schedule for low, medium, and high loaded mesocosms in Subtask 5v.

	Loading schedule (cm/day)				Loading schedule (cm/day)		
Week	**Low**	**Medium**	**High**	**Week**	**Low**	**Medium**	**High**
1*	0	0	0	21	13.2	26.4	66
2	0	0	0	22	13.2	26.4	66
3	22	44	110	23	44	88	220
4	22	44	110	24	44	88	220
5	4.4	8.8	22	25	0	0	0
6	4.4	8.8	22	26	0	0	0
7	0	0	0	27	26.4	52.8	132
8	0	0	0	28	26.4	52.8	132
9	13.2	26.4	66	29	8.8	17.6	44
10	13.2	26.4	66	30	8.8	17.6	44
11	0	0	0	31	35.2	70.4	176
12	0	0	0	32	35.2	70.4	176
13	0	0	0	33	6.6	13.2	33
14	0	0	0	34	6.6	13.2	33
15	26.4	52.8	132	35	11	22	55
16	26.4	52.8	132	36	11	22	55
17	0	0	0	37	22	44	110
18	0	0	0	38	22	44	110
19	17.6	35.2	88	39	0	0	0
20	17.6	35.2	88	40**	0	0	0

* Beginning week for Pulse Loading experiment: Feb. 20, 2000

** Expected week for terminating Pulse Loading experiment: Nov. 20, 2000

Effects of Pulsed Loadings on Phosphorus Retention

For this "pulsing" experiment, weekly total P results are an average of two grab samples, while SRP concentrations represent a composite of two grab samples per week. During this period, the pulsed HLR for the low and medium hydraulically loaded mesocosms varied from 0 to 44 cm/day. Under this loading regime, the effluent SRP concentrations continuously remained near the detection limit (2 µg/L) (Table 3). By contrast, the effluent region of the highest loaded mesocosms, which received a HLR from 0 to 110 cm/day, did have measurable SRP levels, even during the first two weeks when there was no inflow.

The highest effluent SRP concentrations during the quarter coincided with the highest HLR (110 cm/day). These effluent SRP concentrations were comparable to the effluent SRP levels for the control (non-pulsed) mesocosm receiving a constant HLR of 55 cm/day (Table 3). This indicates that wide HLR variation inherent in pulse loading did not, in itself, reduce the SRP removal efficiency of the SAV mesocosms.

Although removal of total P by the SAV pulsed mesocosms was less complete than SRP removal, it did vary consistently among the three HLR treatments (Table 3). For example, average weekly effluent total P concentrations for the pulsed low HLR mesocosms ranged from 17 to 41 µg/L (s.d. = 7.8) over the ten-week period. The effluent concentrations for the pulsed medium HLR mesocosms were higher (25–61 µg TP/L, s.d. = 5.3). There were sharply elevated total P concentrations in the high HLR mesocosms during the period of highest loading (110 cm/day), as well as during the second period of no flow (weeks 7–8), compared to the rest of the period of record (Table 3).

Figure 5 presents the effluent total P concentrations from the twice-weekly grab sampling of the low, medium, and high HLR mesocosms during the ten weeks of the reporting period. The relative flow rates (relative to the historical, constant flow rates of 11, 22, and 55 cm/day) are also shown. These data suggest that pulsed loading is not markedly affecting the outflow quality of the low and medium HLR mesocosms. By contrast, the total P concentrations exiting the high HLR mesocosms are strongly influenced both by the high hydraulic and P loading pulses as well as by the "no-flow" periods. Although we did not actually document bloom-

forming species until the end of May, we suspect that microalgae blooms occurred under the stagnant conditions (no-flow periods) in the highly loaded mesocosms during this quarter, which in turn could contribute to an increase in water column TP concentrations.

Table 3. Mean soluble reactive and total P concentrations (µg/L) in the effluent of duplicate low, medium, and high HLR (cm/day) treatment mesocosms under five bi-weekly hydraulic inflow regimes. The control mesocosms (one per treatment) represent mesocosms from the Long-Term Monitoring (Subtask 5iv) that received a constant HLR.

		Pulsed Mesocosms								
		Low			**Medium**			**High**		
Week	**Date (2000)**	**HLR**	**SRP**	**TP**	**HLR**	**SRP**	**TP**	**HLR**	**SRP**	**TP**
1	Feb 20–26	0	2	17	0	2	27	0	11	54
2	Feb 27–Mar 4	0	2	17	0	4	33	0	8	48
3	Mar 5–11	22	2	23	44	3	30	110	21	72
4	Mar 12–18	22	2	20	44	3	40	110	36	88
5	Mar 19–25	4.4	3	22	8.8	4	29	22	17	64
6	Mar 26–Apr 1	4.4	2	20	8.8	2	25	22	10	56
7	Apr 2-8	0	2	20	0	2	28	0	5	93
8	Apr 9-15	0	2	37	0	2	61	0	6	160
9	Apr 16-22	13.2	3	26	26.4	3	30	66	6	78
10	Apr 23-29	13.2	3	41	26.4	2	34	66	5	65
		Control (Non-Pulsed) Mesocosms								
		Low			**Medium**			**High**		
Week	**Date (2000)**	**HLR**	**SRP**	**TP**	**HLR**	**SRP**	**TP**	**HLR**	**SRP**	**TP**
1	Feb 20–26	11	<2	21	22	2	21	55	11	54
2	Feb 27–Mar 4	11	2	31	22	2	25	55	20	56
3	Mar 5–11	11	<2	30	22	<2	27	55	16	64
4	Mar 12–18	11	2	22	22	2	27	55	23	79
5	Mar 19–25	11	2	23	22	4	23	55	42	90
6	Mar 26–Apr 1	11	2	20	22	2	22	55	19	73
7	Apr 2-8	11	2	24	22	2	191	55	8	120
8	Apr 9-15	11	2	22	22	<2	30	55	6	51
9	Apr 16-22	11	5	34	22	2	29	55	16	46
10	Apr 23-29	11	3	64	22	3	39	55	6	46

The ultimate source of the released P likely is the sediment. Higher P release rates therefore would be expected from the more heavily loaded high HLR mesocosms than from the two lower HLR treatments. Results of our most recent research provide direct evidence of the

importance of previous P loading history as well as the ambient environmental conditions on sediment P release. These data will be presented in a later report.

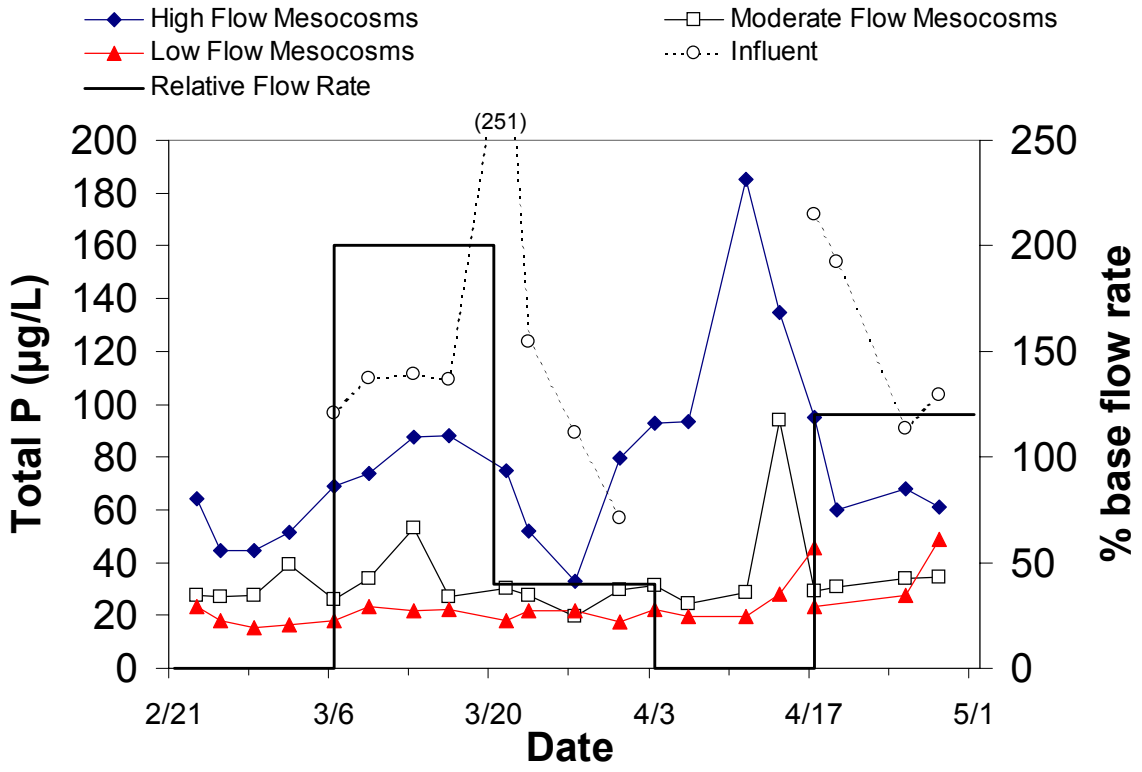

Figure 5. Influent and effluent total P concentrations in the pulse-loading mesocosms under five bi-weekly hydraulic inflow regimes. The relative flow rate represents increase or decrease in the pulsed loading rate with respect to the historical, constant flow rates of 11, 22 and 55 cm/day, respectively, for the low, medium and high flow rate treatments.

Phosphorus Export from Limerock Beds after Resumption of Flows

As an additional component of the pulse-loading study, we investigated the potential for P export from the LR beds immediately following resumption of inflow after a "no flow" period. During a "no flow" period of two weeks or more, the residual interstitial water within the limerock may stagnate, and even evaporate if the dry-out period is long enough. We therefore designed our sampling scheme to capture any "first flush" effects by sampling the LR bed effluent at frequent intervals (ΔT = 1, 3, 21.5 hours) immediately upon resumption of flow. Only short (1.5 day) and moderate (3.5 day) HRT process trains were sampled in this experiment.

On March 6, flows were resumed after two weeks of no flow. Specific conductance values indicated a build up of salts within the LR columns during the no flow period, followed by a rapid decrease within the first 24 hours after the resumption of flow (Table 4). Total P and DOP concentrations in the LR bed effluents were variable but peaked in the ΔT=3-hour samples (Table 4). SRP concentrations decreased after the "one hour" sample following flow resumption. Particulate P (PP) levels increased during the initial 24 hours subsequent to the no flow period (Table 4).

In this experiment we observed substantial differences between treatments with respect to the export of P species. Limerock bed effluent from the high HLR mesocosms consistently yielded higher concentrations of P species, alkalinity and specific conductance than the LR beds downstream of the moderate HLR mesocosms (Table 4).

Table 4. Water quality characteristics of limerock (LR) effluent during the first 21.5 hours of flow on March 6, 2000, following a two-week period of no flow. The pulsed loading data are compared to the limerock effluents from the non-pulsed mesocosm experiment during the February-April 2000 quarter. Each value from the pulsed loading is an average of duplicate LR bed effluents while the non-pulsed loading values represent an effluent from a single LR bed.

Loading	Δ Time (hrs)	SRP (µg/L)	TP (µg/L)	TSP (µg/L)	DOP (µg/L)	PP (µg/L)	Alkalinity (mg $CaCO_3$/L)	Sp. Cond. (µS/cm)
Pulsed High Loading	1.0	37	53	52	15	1	213	943
	3.0	36	75	53	17	22	158	842
	21.5	20	62	31	11	31	133	669
Non-Pulsed High Loading		**31**	**46**	**42**	**11**	**4**	**209**	**898**
Pulsed Moderate Loading	1.0	26	40	35	9	5	210	940
	3.0	19	47	36	16	11	143	831
	21.5	7	33	15	8	18	133	631
Non-Pulsed Moderate Loading		**11**	**21**	**19**	**9**	**2**	**188**	**884**

Generally, initial TP and SRP concentrations exiting the LR beds within the first 21.5 hours were elevated compared to the concentrations measured in the non-pulsed (i.e., constant flow rates) Long-Term Monitoring LR beds (Table 4). Since the duration of the "first flush" after a period of no flow appears to be brief, the overall, long-term impact on the total mass of P exported

would likely be minor. However, P concentrations within the "first flush" may be sufficiently high to warrant preventative measures, such as recycling the LR effluent to the SAV cell(s) for further treatment prior to final discharge.

Sequential SAV/LR Systems and Cattail Mesocosms (Subtask 5vi)

Sequential SAV/LR Systems

As noted in previous reports, the *Chara* in the sequential (deep followed by shallow) SAV/LR systems at the NATTS exhibited a marked decline during 1999. The following is a brief history, based on field notes and algal taxonomic efforts, of the decline of *Chara* populations in these mesocosms. Our first recorded observation was on December 1, 1999, where *Chara* had been displaced by a filamentous green alga in some areas of the SAV-2 mesocosms. The majority of the area of the mesocosm at this time was open water. Since the *Chara* population had already declined considerably by then it is likely that the population was on the decline several months prior, which would coincide with the reduced P removal effectiveness. Indeed, phytoplankton surface scum (*Cystodinium bataviense* and *Cosmarium* sp.) was observed in both the deep (0.8 m) and shallow (0.4 m) mesocosms of replicate no. 1 as far back as June 16, 1999. In a separate mesocosm (SAV-2), thick surface mats of cyanobacteria (*Lyngbya* sp. and *Oscillatoria* sp.) were prevalent on July 7, 1999, persisting as a less dense biomass through August 12, and at least until September 21, 1999. A "*Glenodinuim*-type" dinoflagellate was present as light surface scum in mesocosm SAV-SD-2 on August 12, 1999.

Both the phytoplankton scum and filamentous algae continued to inhabit some of the mesocosms in February 2000, which also coincided with the observed *Chara* mortality. *Lemna* also invaded some of the mesocosms during this time (February – March 2000). Sulfide odors from the decomposing *Chara* were noted in some of the mesocosms in May 2000, along with floating filamentous and non-filamentous algae.

Despite the mortality of *Chara*, which was the dominant SAV species in these tanks, we have continued monitoring of the sequential treatment train which consists of a 0.8 m deep SAV mesocosm; a 0.4 m deep mesocosm; and a final LR bed. The overall HRT of this system is 5 days. Because of wide variations in post-BMP inflow total P concentrations during the quarter

(50-200 µg/L), the deep (0.8 m) SAV mesocosms provided outflow total P concentrations ranging from 50-100 µg/L (Figure 6). Significant P removal occurred in both of the shallow (0.4 m) mesocosms, where effluent total P concentrations averaged less than 50 µg/L. Total P removal was not as high in replicate number two, which may be due to the more pronounced senescence of *Chara* in that system. Further total P concentration reductions within the LR beds ranged from 3 to 21 µg/L during the three-month period (Table 5).

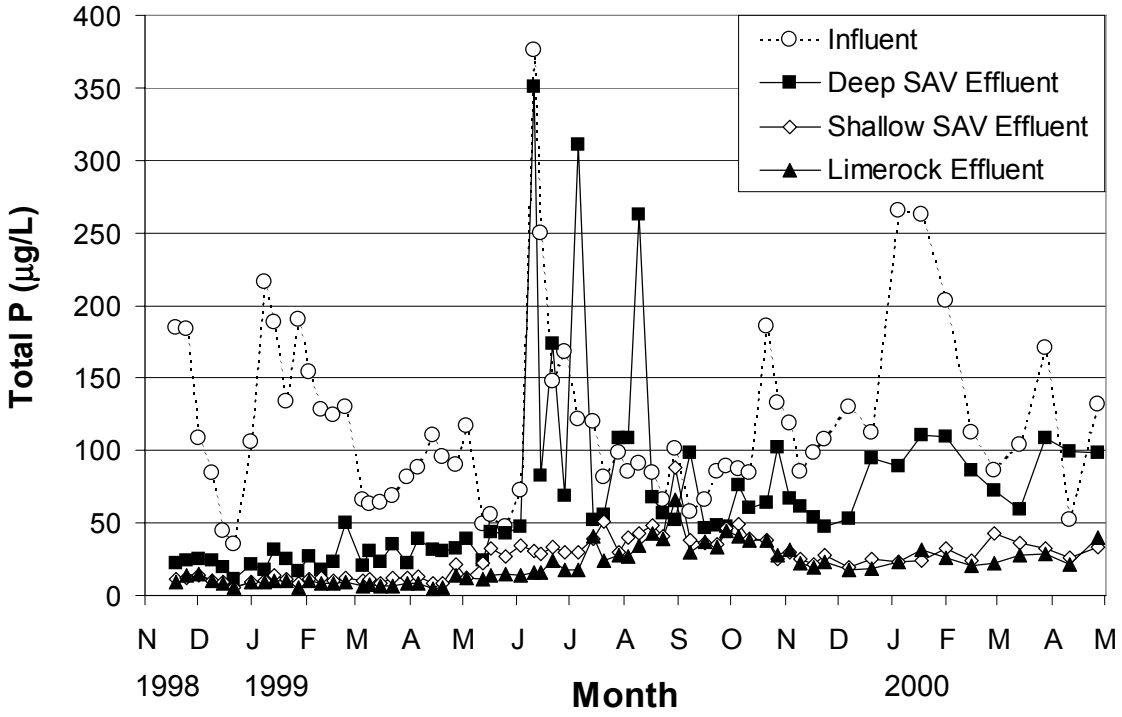

Figure 6. Weekly total phosphorus concentrations at four locations in the sequential system treatment train from November 18, 1998 to May 1, 2000.

Table 5. Mean total P concentrations and pH for four replicate locations in the sequential treatment train (deep SAV →shallow SAV →LR bed) during February-April 2000. These data represent two-week composites of weekly grab samples.

	pH		TP (µg/L)	
	Rep 1	**Rep 2**	**Rep 1**	**Rep 2**
Inflow	7.88	7.93	109	109
Deep SAV Mesocosm Effluent	8.51	8.80	89	86
Shallow SAV Mesocosm Effluent	8.96	8.89	20	45
Limerock Bed Effluent	8.44	8.12	15	39

Cattail Mesocosms

Wetlands dominated by emergent macrophytes such as cattails may function differently than wetlands dominated by SAV, since SAV directly enhances both photosynthesis and nutrient uptake within the water column. An increase in water column photosynthesis leads to an increase in daytime pH, which, in turn, can result in a chemical immobilization of P in hard waters by the coprecipitation with calcium carbonate. As part of this study, we are testing the hypothesis that P removal is more efficient in SAV communities than in a cattail community when the inflow waters have moderately high concentrations of P (50–300 µg/L) and are high in hardness.

Beginning in mid-February 2000, we sampled synoptically the influent and effluent waters of the two cattail and three shallow depth SAV mesocosms located at the NATTS. The SAV community was approximately 6 months older than the cattails; monitoring started in mid-July 1998 and end of December 1998 for the SAV and cattail mesocosms respectively. These two types of plant communities had equivalent water column depths (0.4 m), HLRs (10 cm/day) and HRTs (3.6 days). Weekly grab samples from both sets of mesocosms were composited over a two-week period before being analyzed for TP, SRP, TSP, alkalinity, and Ca concentrations. Specific conductance was also measured.

Data collected during this quarter indicate that the P removal efficiencies for all three P fractions (SRP, TSP, and TP) were higher for the SAV mesocosms than either of the duplicate cattail mesocosms (Table 6). In addition, the pH was higher, and the alkalinity concentrations lower, for the submerged community, indicating that more calcium carbonate precipitation was occurring within the SAV system. Although more data are needed before definite conclusions can be drawn regarding the relative P removal efficiencies of cattail and SAV communities, it appears from this data set that SAV wetlands exhibit superior P removal processes.

Table 6. Comparisons between cattail and SAV dominated mesocosms at the North Advanced Treatment Technology Site in the removal of SRP, TSP, TP and alkalinity during February-April 2000. All data represent two-week composites of weekly grab samples except pH, which is an average of grab samples.

Station	pH	SRP (µg/L)	TSP (µg/L)	TP (µg/L)	Alk (mg $CaCO_3$/L)	Sp Cond (µmho/cm)
Inflow	7.92	65	86	106	206	944
Cattail Effluent Rep 1	7.47	34	51	79	205	936
Rep 2	7.69	16	27	52	206	943
SAV Effluent*	9.32	4	15	31	143	826

* An average of three replicate tanks each sampled as weekly grabs and subsequently composited over a two-week period from February 8 to March 28, 2000. From March 28 to April 30, 2000, only one (SD-2) of the replicate tanks was sampled at the same frequency as before.

Long-Term Phosphorus Removal in Shallow Low Velocity SAV/Periphyton/Limerock Systems (Subtask 5vii)

We have operated shallow SAV/periphyton raceways at the South Advanced Treatment Technology Site since July 1998. On February 4, 2000, we doubled the HLR to the 0.09 m deep SAV/periphyton raceways from 11 to 22 cm/day. At about the same time, the inflow (STA-1W outflow) total P concentrations also doubled (Figure 7), resulting in a four-fold increase in the total P loading to the raceways.

In response to the higher loading, the total P concentrations in the effluents from the raceways and LR beds trended higher. During March, the raceways maintained the effluent total P concentrations at ~20 µg/L, levels first observed in February after the hydraulic loading was doubled. Prior to that flow change, effluent concentrations had averaged 10 µg TP/L (Figure 7). Phosphorus effluent levels near (and occasionally above) the influent concentrations occurred in April, even though the HLR was returned to 11 cm/day beginning April 11, 2000. Total P concentrations in the limerock effluent, which also trended higher in the three-month reporting period, continued to provide additional P removal (except on two occasions in April).

The SRP concentration dropped from 5 µg/L to below the method detection limit (2 µg/L) in the first quarter of the raceway, and remained low in the effluent (Table 7). Because of this decline in SRP, effluent total P consisted entirely of dissolved organic P (DOP) and PP fractions.

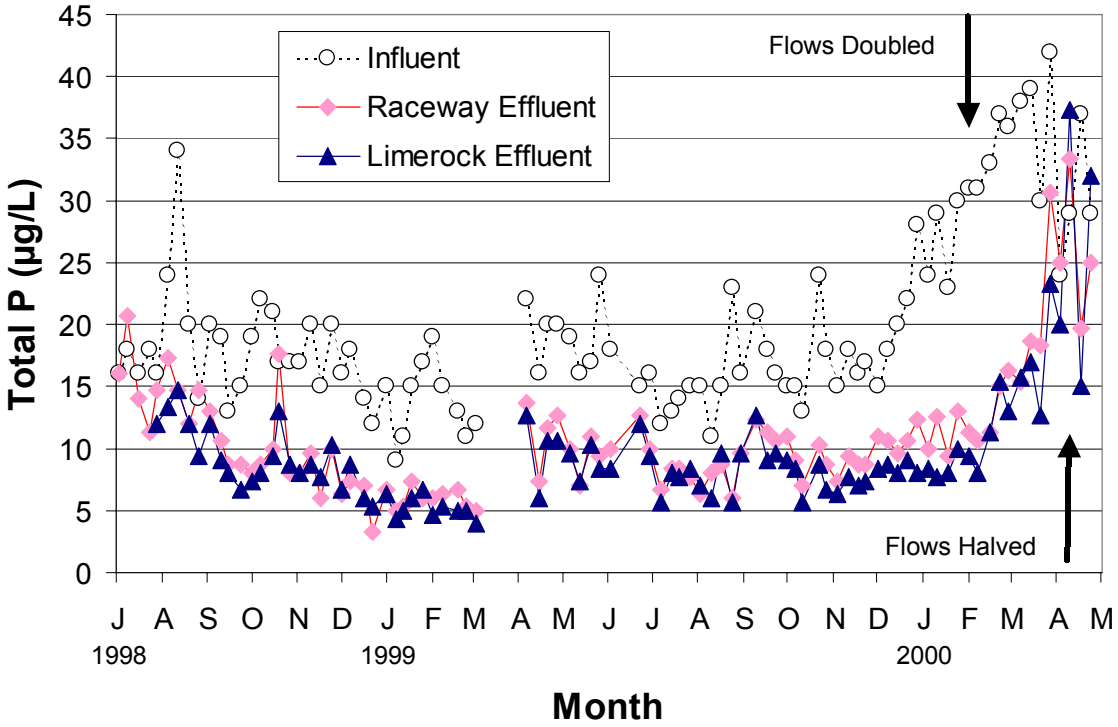

Figure 7. Total P concentrations in the influent and effluent of a shallow, low velocity, SAV/periphyton raceway and in the effluent of the subsequent limerock bed. The first arrow denotes an increase in hydraulic loading from 11 to 22 cm/day and the second arrow marks the return to 11 cm/day.

Table 7. Soluble reactive phosphorus concentrations (µg/L) at four stations along the shallow, low velocity raceway gradient during the February–April 200 quarter. Values represent the average of weekly grabs in the replicate raceways.

Date	Influent	1st Quarter	Midpoint	Effluent
March 21	5	2	2	2
April 3	5	2	2	2
April 10	2	<2	<2	<2
April 17	3	2	2	2
April 24	3	2	2	2
Average	**4**	**2**	**2**	**2**

Growth of SAV in Post-STA Waters on Muck, Limerock and Sand Substrates (Subtask 5ix)

In this study, SAV is being cultured on muck, limerock, and sand substrates in mesocosms at the South Advanced Treatment Technology Site. On February 4, 2000, we decreased the flow to the substrate mesocosms in order to increase the HRT (from 1.3 to 2.6 days) and to reduce the P loading. However, it should be noted that the inflow total P concentrations doubled at approximately the same time that flows were reduced to the mesocosms, so the effects of the longer hydraulic residence time (HRT) do not necessarily correspond to a reduced P loading (Table 8, and Figure 8).

During February and the first part of March, the duplicate mesocosms underlain with muck substrate outperformed mesocosms containing the other two substrates (Figure 8). However, P removal in the muck substrate mesocosms was comparable to those containing the other substrates from mid-March to the end of April 2000.

Table 8. Mass P loadings to substrate mesocosms during periods of different hydraulic loading rates through April 30, 2000.

Date	HLR (cm/day)	Mean Inflow TP (µg/L)	TP Loading (µg/day)
7/8/1999 – 2/3/2000	30.0	18	117
2/4/2000 – 4/10/2000	15.0	35	114
4/11/200 – 4/30/2000	7.5	32	53

We presently believe there are two reasons why there are only slight between-treatment performance differences among the three substrates deployed at the SATT site:

1. Although SAV biomass varies considerably among the substrates (higher for the muck and lower for the limerock and sand), the lack of significant differences in P removal performance may be more a function of the influent P speciation than the treatment environment. In other words, the particulate and dissolved organic P may not be biologically available.
2. None of the substrates received, nor have they had time enough to accrue, a layer of calcareous marl sediment such as is common in Cell 4 and the Everglades. We believe

that it is possible that the presence of this layer may have an effect on the P removal processes within each type of substrate in a differential manner. For example, a marl substrate covering the muck may assist in sequestering the P mineralized from decomposing SAV, which is most abundant in the muck substrate tanks.

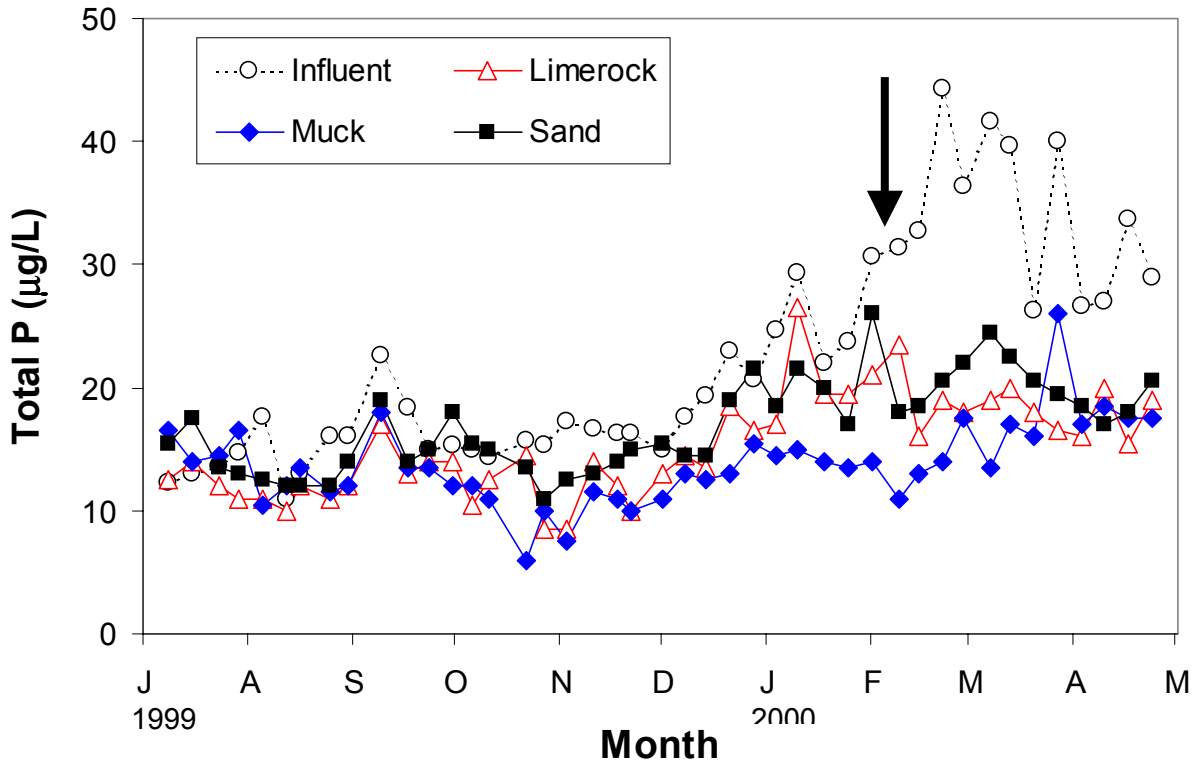

Figure 8. Total P concentrations in the influent (n=3) and the effluents (n=2 per treatment) of SAV mesocosms established on limerock, muck and sand substrates. The arrow denotes a two-fold decrease in flow on February 2, 2000.

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Task 6. Test Cell Investigations

During the February-April quarter, we interrupted the water quality monitoring in three of the four test cells in order to install limerock berms (NTC-15 and STC-9) and to control the aquatic plant *Hydrilla* (STC-4 and STC-9). NTC-1 was routinely monitored throughout the quarter. Additionally, on March 21, 2000, prior to draining the test cells for LR berm installation, we conducted an internal sampling in each test cell, examining SAV distribution and water quality gradients. The details of these efforts are discussed below.

Test Cell Internal Biomass and Water Quality Characteristics

On March 21, 2000, biomass samples were collected near the center of each test cell at the first, second, and third quartile distances from the inflow manifold. Only one biomass sample, retrieved from a 0.09 m^2 area, was taken at each location in each test cell, except at STC-9 where duplicate samples were obtained at each station.

Based on this cursory survey, *Chara* sp. dominated the aquatic plant community in each of the four test cells, with *Hydrilla* also being prominent in the south test cells (Table 9). A biomass density gradient existed in the north test cells where the highest SAV density occurred at the station closet to the inflow and the lowest was located at the station furthest from the inflow. Among the test cells, the highest aggregate biomass density was found in STC-9.

Because of the limited number of sampling stations (3) per cell, our biomass inventory can only be construed as a brief survey and not as a quantitative assessment of the standing crop of SAV. For example, field notes collected a week prior to the sampling indicated that considerably more *Hydrilla* was present in the two south test cells than was recovered in the survey. Also, expansive stands of *Najas* were present in NTC-15, but none was present in the three stations that were sampled within that test cell.

The same stations from which biomass samples were collected also served as the location for surface water grabs, which were taken prior to the biomass sampling. A 500-mL Nalgene

sample bottle (acid-cleaned) was placed 0.1 m under the water surface and allowed to completely fill. Aliquots from the 500-mL bottle were subsequently dispensed into the appropriate analyte (SRP, TSP, TP, Ca, alkalinity) bottle. Dissolved oxygen concentration was measured *in situ*; pH readings were performed on a subsample withdrawn from the 500-mL collection bottle.

Most of the changes in water quality constituents occurred within the first quartile distance along the length of the test cells (Table 10). The most notable change was in the SRP concentration at the north test cells, where influent concentrations of 59 and 61 µg/L decreased to 2 µg/L at the first sampling station, one fourth the length of the cell. The decrease in the total P concentrations was less marked along the longitudinal gradient in NTC-1 than NTC-15, most likely due to the lower *Chara* biomass in NTC-1 compared to NTC-15 (Table 9 and Table 10).

Standing crop biomass differences also probably explain the lower removal rates for alkalinity and calcium in NTC-1, in contrast to NTC-15. The photosynthetically induced rise in pH was considerably lower in NTC-1 than in NTC-15 (Table 10), thereby diminishing the extent of calcium carbonate precipitation. The reduced calcium carbonate precipitation that occurred in NTC-1 also explains the higher specific conductance values in that cell compared to NTC-15.

Spatial differences in SAV distribution between NTC-1 and NTC-15 likely account for the observed water column dissolved oxygen (DO) characteristics of the two test cells. The DO concentrations were lower at the surface in NTC-1, where *Chara* was absent, than in NTC-15, where SAV was "topped out". Likewise, dissolved oxygen concentrations were highest in the bottom waters of NTC-1, where the *Chara* was abundant. The opposite was true for DO concentrations in NTC-15: low levels were measured in the bottom waters because of shading from the overlying SAV canopy.

Table 9. Submerged aquatic vegetation characteristics along the longitudinal axes of two north test cells (NTC-1 and NTC-15) and two south test cells (STC-4 and STC-9) on March 21, 2000.

Cell	Distance from inflow (%)	Genus	Bulk Wet Weight (g)	Bulk Dry Weight (g)	Dry/Wet Ratio	Biomass Density (g dry/m^2)
NTC-1	25	*Chara*	283.3	41.4	0.146	450
	50	*Chara*	220.4	29.8	0.135	324
	75	*Chara*	214.7	30.3	0.141	329
NTC-15	25	*Chara*	642.9	90.7	0.141	986
	50	*Chara*	452.9	76.8	0.170	834
	75	*Chara*	319.0	47.5	0.149	515
STC-4	25	*Chara*	360.5	53.2	0.148	578
	50	*Chara*	317.1	53.1	0.167	576
	75	*Chara*	194.3	22.1	0.114	240
	25	*Hydrilla*	12.1	1.0	0.079	10.4
	50	*Hydrilla*	11.1	0.7	0.059	7.2
STC-9	25	*Hydrilla*	610.7	76.4	0.125	830
	50	*Chara*	724.7	92.7	0.128	1007
	75	*Chara*	679.1	96.4	0.142	1047

Table 10. Concentration gradients for water quality parameters along the longitudinal axes of two north test cells (NTC-1 and NTC-15) and two south test cells (STC-4 and STC-9) on March 21, 2000. Grab samples (one from each location) were collected between 1100 and 1740 hours.

Cell	Sample Location	SRP (µg/L)	TP (µg/L)	TSP (µg/L)	Alkalinity ($mgCaCO_3/L$)	Calcium (mg/L)	DO Top (mg/L)	DO Bot. (mg/L)	pH	Temp (°C)	Sp. Cond (µS/cm)
NTC-1											
	Influent Manifold	59	107	77	198	56.0			7.97	29.5	869
	1st Quarter	2	33	10	192	48.2	10.4	20.0	8.20	30.7	860
	Midpoint	2	36	11	192	47.6	10.0	17.8	8.30	30.9	872
	3rd Quarter	4	57	10	188	47.6	11.0	13.0	8.39	32.2	832
	Effluent Weir	2	25	10	178	43.7			8.35	30.0	825
NTC - 15											
	Influent Manifold	61	88	80	200	62.7			7.89	26.5	869
	1st Quarter	2	16	8	116	24.0	18.8	1.4	9.55	31.2	720
	Midpoint	2	17	8	116	24.7	14.4	5.2	9.25	31.0	723
	3rd Quarter	2	19	10	118	23.9	15.2	6.7	9.22	30.7	715
	Effluent Weir	2	17	10	140	31.6			8.58	28.0	754
STC - 4											
	Influent Manifold	5	25	14	236	62.6			8.01	25.2	1016
	1st Quarter	2	17	8	130	26.4	20.0	20.0	9.51	31.1	712
	Midpoint	2	13	8	122	22.8	19.8	3.3	9.74	31.5	682
	3rd Quarter	2	13	8	125	21.8	20.0	10.0	9.54	31.1	697
	Effluent Weir	2	14	10	130	25.6			8.91	25.0	708
STC - 9											
	Influent Manifold	5	20	14	238	61.7			7.97	24.7	983
	1st Quarter	2	21	10	155	32.8	14.5	6.5	9.01	27.9	821
	Midpoint	2	20	10	146	29.9	13.0	1.5	8.97	27.4	777
	3rd Quarter	2	22	11	147	29.0	14.2	5.0	9.03	27.1	788
	Effluent Weir	2	22	11	157	31.0			8.47	25.3	797

During this test cell survey, three of the test cells were being operated at a depth of 0.8m, while one test cell, STC-4, had a water column depth of only 0.4m. For the south test cells, which typically provided a lower P removal rates than the north test cells, the shallower cell (STC-4) exhibited a more dramatic removal of total P, alkalinity and Ca than the deeper cell (STC-9). Whether the improved removal efficiencies in TP, alkalinity, and Ca for STC-4 were due to the shallower depth in STC-4 or to differences in biomass characteristics cannot be determined without further study.

Test Cell Phosphorus Removal Performance

Since the fall of 1999, we have subjected the north and south test cells to wide variations in hydraulic loadings, largely to accommodate our hydraulic tracer investigations and cell modifications. Despite these operational variations, we have collected preliminary, "baseline" TP removal data on a weekly basis. Although both NTC-1 and NTC-15 were maintained at the same depth and HRT during February and March 2000, the latter cell exhibited a higher P removal efficiency (Figure 9). This is probably due to the reduction in *Chara* biomass we first observed in NTC-1 after the HLR was quadrupled during December 1999 (for the second tracer study), and which was later confirmed in our March SAV survey (Table 9).

We noticed in March during our SAV survey that the *Chara* beds within NTC-1 were not fully "topped out" like they had been prior to the tracer study. The biomass density was found to be only about one-half that in NTC-15 (Table 9). Whether the reduction in the *Chara* biomass was due to the increased P loadings, higher flow rates, or decreased light penetration that occurred during the 3-4 weeks of the tracer study, or just a natural senescence, is not known. Both higher P concentrations and decreased light penetration have been shown in the literature to negatively affect *Chara* populations (Forsberg 1965; Steinman et al. 1997).

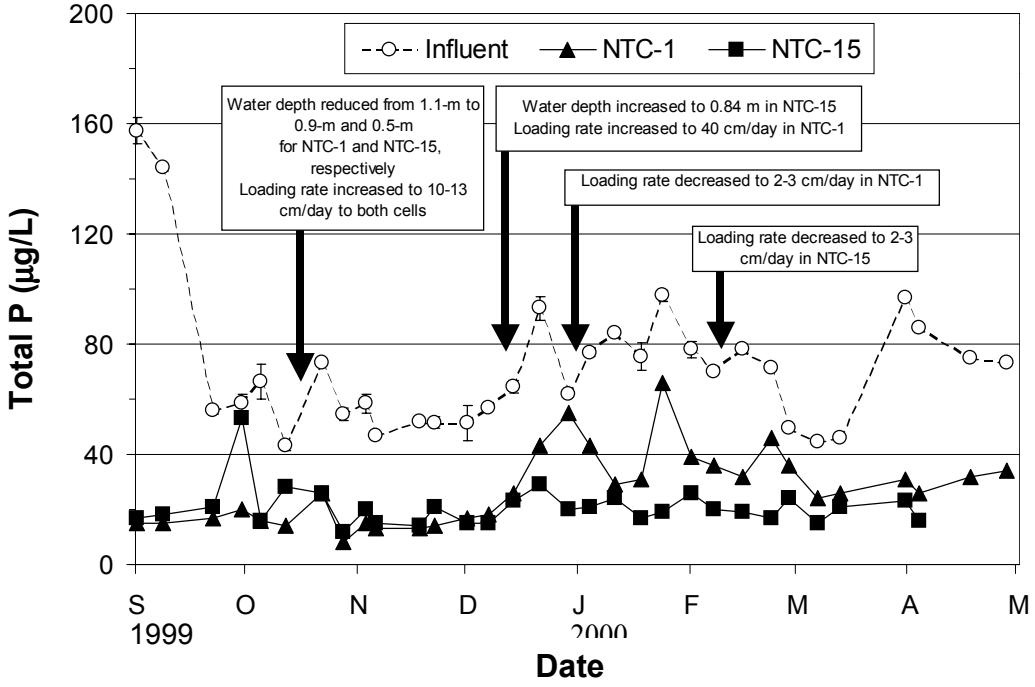

Figure 9. Total phosphorus concentrations in the influent and effluents of north site test cells. Error bars = ± 1 s.d. Sampling was discontinued in April at NTC-15 because of draining in preparation for the installation of a LR berm in the cell.

Both south test cells continued to perform poorly during February and during the first part of March (Figure 10). At that time, we drained both cells for aquatic plant control and installation of a limerock berm (STC-9 only). We have hypothesized in previous reports that the poor P removal may be due to more recalcitrant forms of DOP and PP in the inflows to the south test cells, as compared to the north test cells. The presence of *Hydrilla* in the south test cells also may contribute to this poor performance.

Installation of Limerock Berms

In our Phase 1 project, we demonstrated that filtration of SAV mesocosm effluent through limerock columns was beneficial in removing some fraction of the dissolved organic and particulate phosphorus (DBEL 1999). To investigate this premise at a larger scale, we installed limerock berms in two of our test cells, NTC-15 and STC-9, in April 2000.

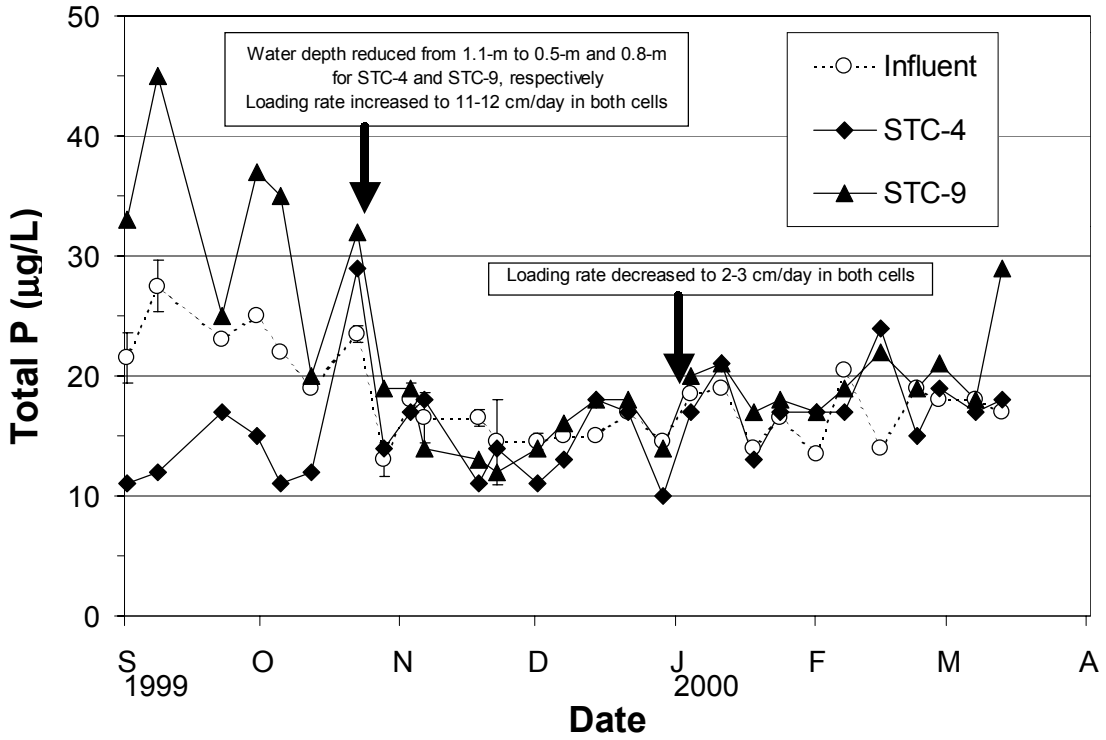

Figure 10. Total phosphorus concentrations in the influent and effluents of south site test cells. Error bars = ± 1 s.d.

The berms are located approximately 88% down the length of the test cells from the inflow. Each berm was constructed directly on top of the existing test cell peat substrate. The foundation of each berm was comprised of two layers of structural geotechnical fabrics, sandwiching 6 inches of limerock base fill material. The foundation served two purposes: to provide a 'snowshoe' effect by distributing the weight of the berm evenly over the peat substrate and to protect the test cell liner during installation and removal. Each berm foundation is approximately 16 feet wide.

The washed limerock comprising the berms is No. 4 Ballast Stone (approximately 1 inch diameter). Each berm is approximately 4 feet tall and 4 feet wide at the top, with sides that taper from the 4 feet top width to the 16 feet foundation width. Each fully installed berm contains approximately 130 yd^3 of limerock.

Assuming an average water depth of 1.3' (40 cm), the mean length of travel in the direction of flow through the berm (berm width) is approximately 14'. For reference, if the test cell HLR was 10 cm/day (HRT = 4 days), the mean residence time in the berm would be about 5 hours, which is comparable to residence times in limerock column studies from our Phase 1 work (DBEL 1999).

References

DB Environmental Laboratories (DBEL). 1999. A Demonstration of Submerged Aquatic Vegetation/Limerock Treatment System Technology for Removing Phosphorus from Everglades Agricultural Area Waters. Final Report submitted to the South Florida Water Management District and the Florida Department of Environmental Protection. West Palm Beach, FL.

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Task 9. Development of Performance Forecast Model

During this quarter, our efforts focused on a detailed analysis of Cell 4 performance for the 1998-1999 calendar years. As evidenced in Table 11, Cell 4 exhibited improved TP removal performance during 1998 and 1999 compared to the period 1995 – 1997. During 1998-1999, effluent concentrations were substantially lower than years previous, while mass removal rates, TP settling rates, and removal efficiencies were all higher than historic averages. The equations used to calculate these parameters were given in our 1st Quarterly Report (DBEL 2000). Note that the values shown for 1999 in Table 11 represent the entire calendar year, whereas the similar table in our 1st Quarterly Report represented only the first half of the year.

Table 11. Summary of Historic Cell 4 TP Removal Performance

	Effluent TP (µg/L)	Mass Removal ($g/m^2/yr$)	Settling Rate, k (m/yr)	Removal Efficiency (%)
1995	21	0.7	28	26
1996	29	1.7	49	31
1997	21	0.7	26	30
1998	14	1.2	44	70
1999	14	1.9	55	61
Average	20	1.2	40	44

The intent of a detailed review of 1998 – 1999 Cell 4 performance was to identify key relationships between Cell 4 inflow parameters (hydraulic loading, TP mass loading, nominal flow velocity) and TP removal performance. In this section, we present an analysis of the 1998 – 1999 effluent TP concentrations and a summary of regressions relationships for TP concentration and TP settling rate (K) with hydraulic loading, mass loading, depth, and velocity. We also present a preliminary assessment of the relative importance of a 'velocity effect' in TP removal for SAV systems.

1998 – 1999 Cell 4 Effluent TP Concentrations

Figure 11 shows a time history of inflow and outflow TP concentrations for 1998 – 1999. The plotted concentrations are from the District's weekly composite TP data collected at the G254

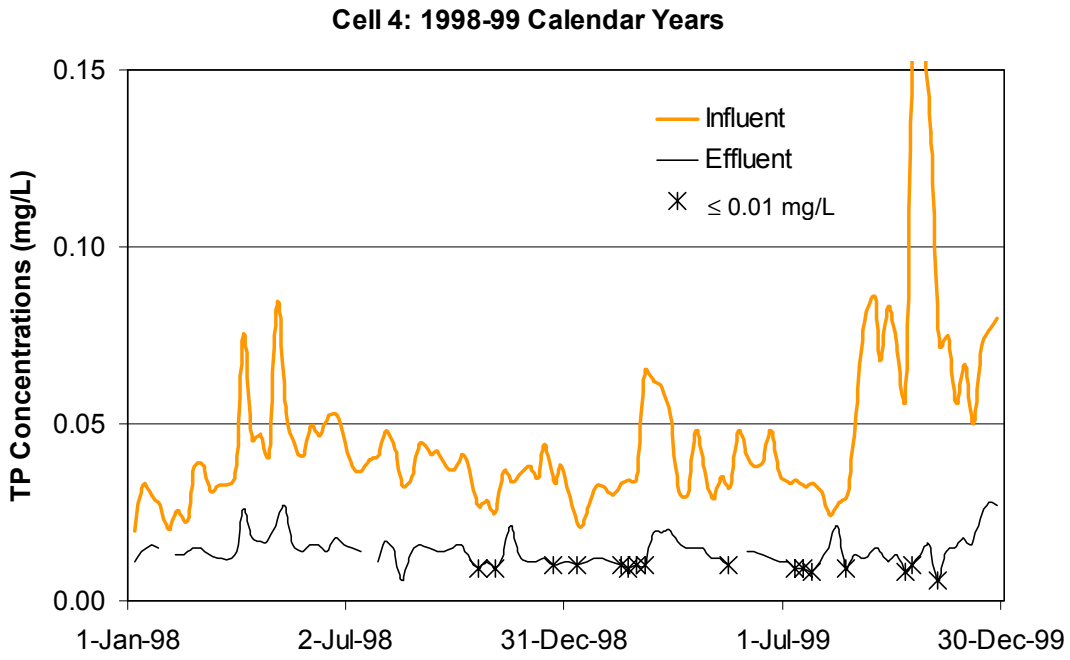

Figure 11. Influent and effluent TP concentrations for 1998 – 1999.

and G256 culverts. Inflow concentrations for the two-year period averaged 46 µg/L and outflow concentrations averaged 14 µg/L. Effluent concentrations were relatively stable during this period, with some evidence of a trend towards rising effluent concentrations beginning December 1999. The Cell 4 effluent TP concentration on December 27, 1999 was 28 µg/L.

Figure 11 also highlights composite samples that measured less than or equal to 10 µg/L TP. In 1998, there were three occurrences (out of 47 reported samples) of ≤ 10 µg/L, whereas in 1999 there were 13 occurrences (out of 51 reported samples). Figure 12 summarizes 1999 performance with a frequency distribution of TP measurements grouped in 5 µg/L increments. In 1999,

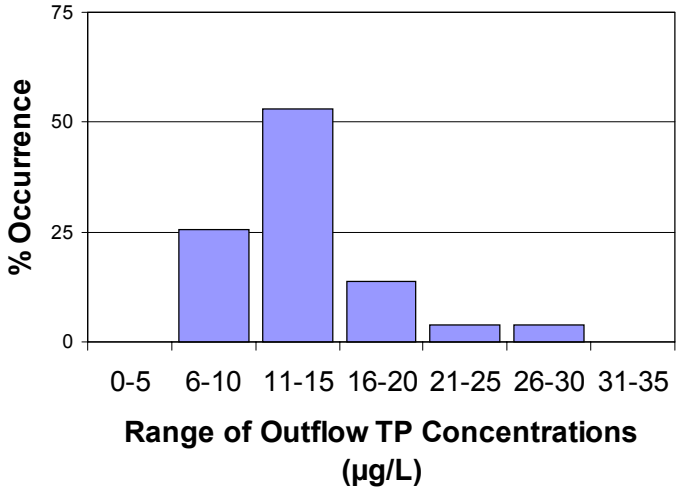

Figure 12. Frequency distribution of effluent TP concentrations for 1999, based on 51 weekly composites.

26% of Cell 4 effluent TP measurements were ≤ 10 µg/L, while 78% of measurements were ≤ 15 µg/L.

Figure 13 and Figure 14 show Cell 4 hydraulic loading rate (HLR) and TP mass loading rate for 1998-1999. The HLR during this period had a range between 0.2 and 33 cm/day, and averaged 12 cm/day. Beginning September 1999, there was a sustained increase in HLR to an average value of 16 cm/day, approximately 33% higher than the average of the previous 20 months. Mass loading was calculated by multiplying the daily HLR by the appropriate weekly composite influent TP concentration. The measured value of TP concentrations from composite samples were assumed to apply to the day collected as well as to the six previous days (composite samples were comprised of 3x daily grabs collected for 7 days). Annualized mass loading averaged 1.5 g/m²/day for the 20 month period of January 1998 through August 1999, and 4.9 g/m²/day beginning September 1999. Mass loading to Cell 4 more than tripled in the last months of 1999. Examination of Figure 11 indicates that increased influent TP concentrations were the most significant cause of increased mass loading. Influent concentrations more than doubled in the last four months of 1999 compared with months previous.

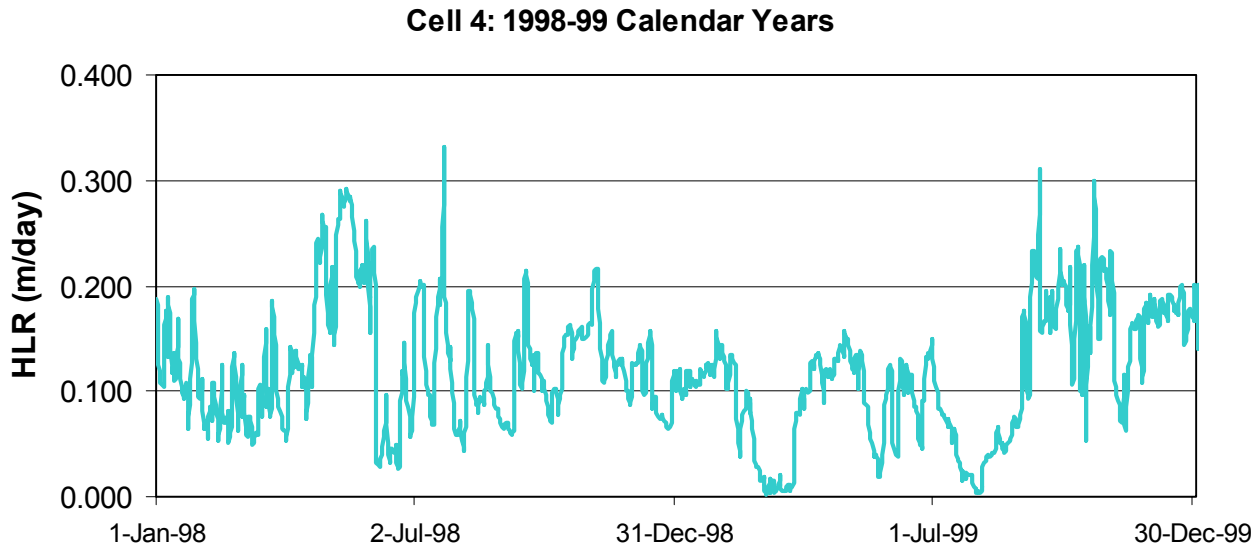

Figure 13. Cell 4 hydraulic loading rate (HLR) for 1998 – 1999.

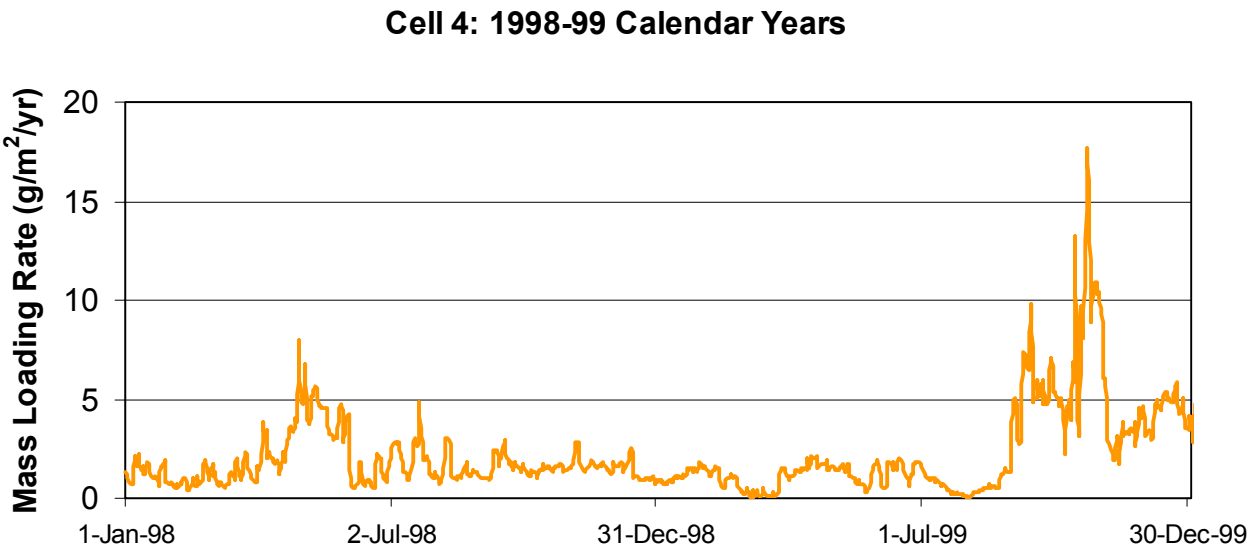

Figure 14. Cell 4 annualized TP mass loading rate for 1998 – 1999.

We believe that the December 1999 rise in effluent concentrations (Figure 11) was a direct result of the near step-function increase to Cell 4 mass loading that began in September 1999 (Figure 4). It is important to note the apparent three-month lag period between increased mass loading (September) and a noticeable response in effluent TP concentrations (December). In fact, during this three month period, there were two occurrences of weekly TP measurements that were ≤ 10 µg/L. This apparent lag between influent loading and effluent discharge could be indicative of a short-term storage capacity within SAV systems and a capacity to 'buffer ' surges in TP loading, up to a limit. Our ongoing pulse-loading experiments on the mesocosm scale will help us further investigate the transitory response of SAV systems to dynamic loads. We will also continue to analyze the Cell 4 data set as it develops, with particular interest on the recovery (relaxation) response in Cell 4 if mass loading returns to a more typical range.

Operational and Water Quality Regression Relationships

We performed regression analyses on the 1998 – 1999 Cell 4 data to determine the relative significance of operational parameters on effluent TP concentration and on the TP settling rate (k). The operational parameters that we analyzed were HLR, mass loading, water depth, and nominal velocity (based on an average Cell 4 width of approximately 700 m). To reduce the

influence of hydraulic retention time on input/output regression analyses, we used weekly average values for all parameters. Weekly average values of HLR, mass loading, depth, velocity, and settling rate were calculated for the same days as composite TP samples were collected (in the District's data set) using the previous week's flow and depth data. Therefore, the regression analyses for 1998-1999 were based on 98 data points (47 from 1998, 51 from 1999) that represented weekly average values of the plotted parameters.

Figure 15 and Figure 16 show typical regression plots for mass loading versus TP concentration and TP settling rate (k), respectively. Table 12 summarizes correlation coefficients (r^2) for the eight regression analyses performed. Note that the K vs. HLR and K vs. mass loading regressions should be interpreted with caution, as there is a potential for auto-correlation in both of these calculations (common variables are used to calculate both parameters in these regressions). As could be expected, direct correlations between operational parameters and effluent TP concentration were weak, while correlations with settling rate were much stronger. In both cases, the most significant factor (highest r^2) influencing TP removal was the TP mass loading rate. HLR was also an important factor. Positive correlations were evident between settling rate and both velocity and depth, but they were substantially weaker than compared to TP mass loading (which, as discussed above, could be artificially inflated due to auto-correlation).

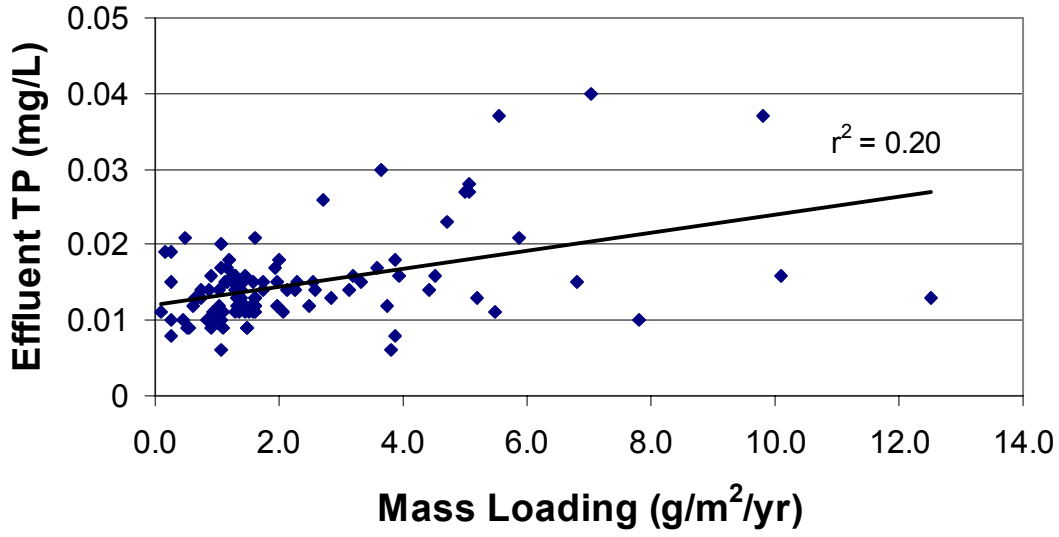

Figure 15. Regression analysis of weekly average (composite) effluent TP concentration with weekly average TP mass loading from 1998 – 1999 data.

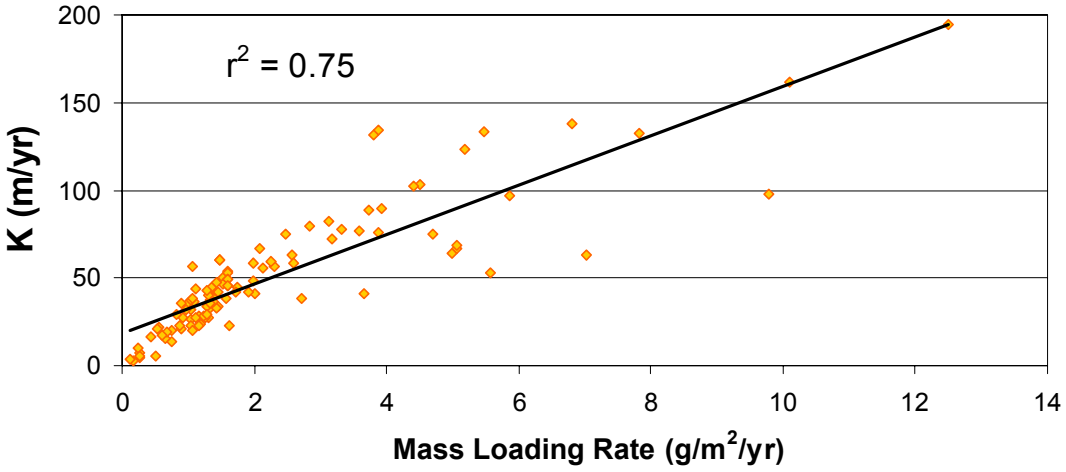

Figure 16. Regression analysis of weekly average TP settling rate (k) with weekly average TP mass loading from 1998 – 1999 data. Note that equations for calculating K and mass loading both contain terms for inflow concentration and hydraulic loading rate. Therefore, auto-correlation is partly responsible for the high correlation.

Table 12. Summary of Cell 4 regression analyses based on 1998 – 1999 Data. Note that K vs. HLR and K vs. mass loading regressions should be interpreted with caution, as there is a degree of auto-correlation in these calculations.

Operational Parameter	Correlation Coefficients (r^2)	
	Effluent TP Concentration	TP Settling Rate (k)
Water Velocity	0.12	0.41
Water Depth	0.001	0.58
HLR	0.14	0.61
TP Mass Loading	0.20	0.75

Relationship Between SAV Performance and Flow Velocity

Some researchers have proposed that treatment performance in SAV systems might increase as a function of flow velocity through the system; this has been termed the 'velocity effect'. The implication is that experimental results at the mesocosm scale, which typically operate with much slower velocities than STA-scale systems, may not be indicative of STA-scale performance. To investigate this premise, we performed a comparative analysis of a 4-month period in the Cell 4 data record (September 1999 - December 1999) to a DBEL experimental

mesocosm that has operated for over 20 months under similar average hydraulic and nutrient loading conditions as the Cell 4 period. Table 13 summarizes this comparison. Nominal flow velocity is approximately two orders of magnitude less in the mesocosm compared to Cell 4, while other operational parameters (HLR, mass loading) are quite similar. Similarities in both effluent concentrations and settling rates (k) between scales suggest that the velocity effect may not be significant in SAV systems. We will continue to investigate the velocity effect in our Phase 2 project with mesocosm scale experiments and with future Cell 4 performance data, as it becomes available.

Table 13. Summary of Cell 4 and Mesocosm 'Velocity Effect' Comparison

	Cell 4: 9/99 – 1/00 (5 month average)	**Mesocosm* (20 month average)**
HLR (cm/day)	18	22
HRT (days)	3.6	3.5
Mass Load ($g/m^2/yr$)	5.6	8.2
Mean Velocity (cm/s)	0.54	0.0015
TP–in (µg/L)	85	102
TP–out (µg/L)	19	29
K (m/yr)	98	101

* moderately loaded (22 cm/day) mesocosm from hydraulic loading study at the NATTS.

References

DB Environmental Laboratories (DBEL). 2000. A Demonstration of Submerged Aquatic Vegetation/Limerock Treatment System Technology for Removing Phosphorus from Everglades Agricultural Area Waters: Follow-on Study. 1st Quarterly Report submitted to the South Florida Water Management District and the Florida Department of Environmental Protection. West Palm Beach, FL.

Task 10. Cell 5 Inoculation and Monitoring

SAV Inoculation Experiments in Cell 5

The most cost-effective approach for rapidly establishing submerged macrophytes in a large-scale SAV-dominated wetland is currently unknown. The western portion of STA-1W Cell 5 was flooded quickly during late spring 1999, and this has created large areas of open water with few emergent macrophytes. It is not known, however, whether these open areas will be rapidly colonized with SAV via natural recruitment, or if SAV inoculation ultimately will be required. The simplest means of SAV inoculation would be to obtain plant material from a nearby donor wetland (e.g., Cell 4), and disperse small aliquots throughout the cell from an airboat. However, the 930 ha western portion of Cell 5 has a large, open fetch, so widely distributed plant fragments could all be swept to one side of the wetland if a strong wind were to blow soon after plant stocking.

Beginning in December 1999, we initiated two experiments to evaluate potential inoculation protocols for SAV in Cell 5. Both techniques involved "weighting" small aliquots of plants prior to stocking. In the first, we placed small, known quantities of the macrophytes *Ceratophyllum*, *Najas* and *Chara* into open mesh bags. Both small mesh (fiberglass screen) and large mesh (polypropylene "citrus" bags) were evaluated. The bags and enclosed plants were weighted with a small brick, and incubated just below the water surface, as well as at the sediment-water interface. The near-surface incubation was performed to determine whether the poor light transmittance in the water column (caused by high organic color levels) was inhibiting SAV growth. For the second technique, a narrow ceramic "ring" was placed over a small "handful" of plants, thereby providing a weight to hold that "cluster" of plants on the bottom. For both experiments, plants were retrieved and wet weight biomass measured at two and four weeks after stocking.

Neither inoculation technique proved successful, primarily because portions of the stocked plants became stressed and died. Even though the plants were loosely stocked in the incubation bags, the plants actually in contact with the bag material either grew poorly or died. The plants directly in contact with the ceramic ring were similarly stressed. Some plants, in particular the

Ceratophyllum that was not directly in contact with the bag or ring, grew well. Overall, *Ceratophyllum* and *Najas* exhibited positive growth in some of the bags, whereas all of the stocked *Chara* died. For *Ceratophyllum* and *Najas*, the large mesh bags were more effective than the small mesh bags in promoting SAV growth, and the plants incubated near the surface grew better than those incubated adjacent to the bottom. Data collected from the internal Cell 5 survey (see below) suggest that water column characteristics, rather than incubation techniques, may have been responsible for the mortality of the *Chara*.

Internal Cell 5 SAV Survey

In order to assess the colonization rate of SAV species into Cell 5, we are performing quarterly assessments of SAV occurrence in the wetland. Early in February 2000, using Global Positioning System (GPS) coordinates, we established a grid of 120 equidistant sampling stations in the western portion of STA-1W Cell 5. We marked each station in the field by driving a labeled PVC pole into the muck substrate. On February 10, we performed the first assessment of SAV occurrence. This work was performed from an airboat. Because of the highly colored waters, vegetation on the bottom was not visible, so we used a garden rake to collect submerged vegetation. We developed a "standard" collection technique, in which the rake was "dragged" along the bottom at two locations adjacent to each sampling site. Collected SAV species were identified, ranked qualitatively in terms of abundance, and returned to the water.

A map of the distribution of vegetation in Cell 5 on February 10, 2000 is provided in Figure 17. Note that this reflects plant species found immediately adjacent to each sampling location, and therefore does not represent vegetation occurring "between" stations. The area of the western portion of Cell 5 is ~ 930 ha, so each of our 120 sampling points is an 'approximation' of a ~8 ha parcel.

Ceratophyllum was found to be the dominant SAV species in Cell 5, occurring in 64 of 120 stations. *Najas* was found in 17 stations, and *Chara* in none. In general, the biomass of SAV at most locations was quite low (only a few plant fragments), reflecting only very recent colonization. The distribution of floating and emergent macrophytes in Cell 5 also is shown in Figure 17. Overall, 98 of 120 stations had some macrophytes present. The northwest region of

the wetland was open water, essentially devoid of macrophyte biomass. Secchi depths during this survey ranged from 0.05 to 0.45m, and averaged 0.25m.

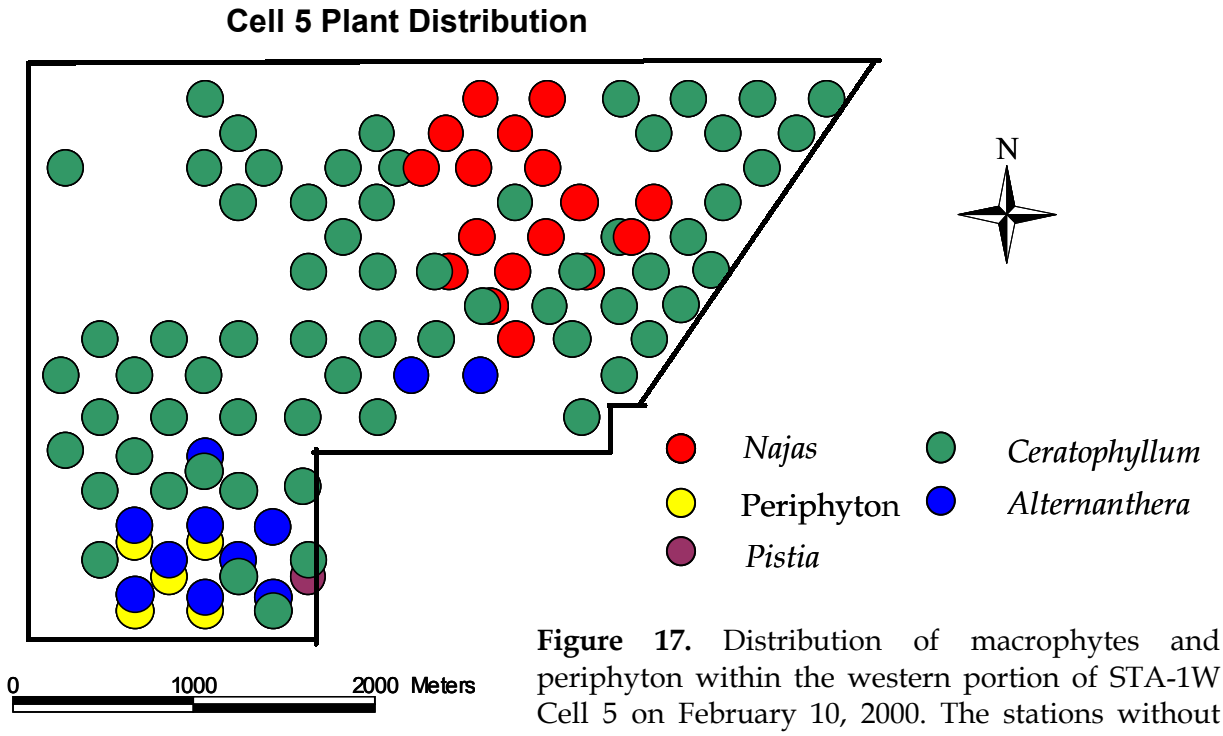

Figure 17. Distribution of macrophytes and periphyton within the western portion of STA-1W Cell 5 on February 10, 2000. The stations without macrophytes are depicted by open spaces.

Two main factors influence the distribution of SAV species in a newly flooded wetland. The first is the availability of SAV propagules, and the second is the suitability of environmental conditions, such as physical and chemical characteristics of the substrate and water column, for SAV growth. Since its startup in early summer 1999, Cell 5 has exhibited high concentrations of both total P and color ($\bar{x}$ = 168 µg P/L and 203 mg CPU/L) in the water column. Based on both our inoculation experiments and the SAV distribution in the cell, these conditions appear detrimental to *Chara*, but favorable for *Ceratophyllum*, and to a lesser extent, *Najas*. In the north bank of test cells, which exhibited better water quality (inflow TP concentrations averaged 84 µg/L from September 1, 1999 to August 4, 2000) upon flooding in 1998, *Chara* immediately grew throughout the cell and became the dominant SAV species.

The Cell 5 monitoring will be continued quarterly through June 2001 to determine how quickly SAV species proliferate throughout the wetland.